Plant and soil microbial responses to drought stress in different ecosystems: the importance of maintaining the continuum

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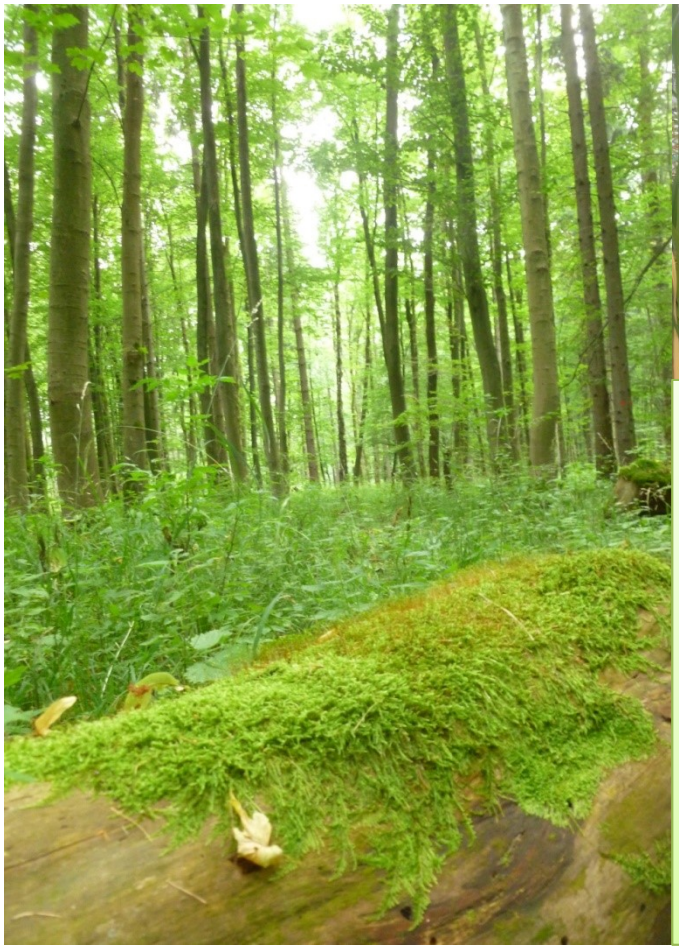

"Twenty years from now you will be more disappointed by the things that you didn't do than by the ones you did do. So throw off the bowlines. Sail away from the safe harbor. Catch the trade winds in your sails.

Explore. Dream. Discover."

Mark Twain

American Writer

For Miezie, my beloved cat <3

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List of abbreviations

%	Percent
%max	Maximum water holding capacity
°C	Degree Celsius
µg	Microgram
µl	Microliter
µmol	Micromole
‰	Per mille
1/D	Inverse Simpson diversity index
^{11}C, ^{12}C, ^{13}C, ^{14}C	Isotopes of carbon
16S rRNA	16S ribosomal ribonucleic acid
A	Chance-corrected within-group agreement
A	Photosynthesis
Ah	Accumulation of humus
ANOVA	Analysis of variance
Bp	Base pair
C	Control or Carbon
cDNA	Complementary deoxyribonucleic acid
cm	Centimeter
CO_2	Carbon dioxide
D	Drought
DD	Drought day
DNA	Deoxyribonucleic acid
DW	Dry weight
E	East
FAME	Fatty acid methyl ester
Frc	Fructose
FW	Fresh weight
g	gram
GHG	Greenhouse gas
Glc	Glucose
g_s	Stomatal conductance
Gt	Gigatonne
h	Hours

H	Heat
H_3PO_4	Phosphoric acid
ha	Hectare
HD	Heat-drought
HSD	Honestly significant difference
HTS	High-throughput sequencing
kg	Kilogram
kPa	Kilopascal
l	Liter
L x W x D	Length x width x depth
LEA	Late embryogenesis abundant
M	Meter
MC	Moisture content
mg	Milligram
min	Minute
ml	Milliliter
MLSA	Multilocus sequence analysis
mm	Millimeter
mM	Millimole
MPa	Megapascal
mRNA	Messenger ribonucleic acid
MRPP	Multi-response permutation procedure
MRT	Mean residence time
n	Number of replicates
N	Nitrogen or North
NA	Natural abundance
NaOH	Sodium hydroxide
NGS	Next-generation sequencing
NMS	Nonmetric multidimensional scaling
NPP	Net primary production
NSC	Non-structural carbohydrates
nt	Nucleotide
O	Oxygen
Ø	Average
OM	Organic matter

OTU	Operational taxonomic units
P	Probability value
P. australis	*Phragmites australis*
PAR	Photosynthetically active radiation
PC	*Phragmites australis* Control
PCR	Polymerase chain reaction
PD	*Phragmites australis* Drought
PLFA	Phospholipid fatty acid
ppm	Parts per million
PVC	Polyvinyl chloride
RH	Relative humidity
RNA	Ribonucleic acid
rpm	Revolutions per minute
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
s	Second
SD	Standard deviation
SE	Standard error
SM	Soil moisture
SOM	Soil organic matter
SSU	Small subunit
Suc	Sucrose
T	Temperature
T. latifolia	*Typha latifolia*
T_{air}	Mean daily air temperatures
TC	*Typha latifolia* Control
TD	*Typha latifolia* Drought
T_{soil}	Mean daily soil temperatures
VPDB	Vienna Pee Dee Belemnite
VWC	Volumetric water content
$\delta^{13}C$	Carbon isotopic composition relative to international standard VPDB
Ψ	Water potential

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Summary

Climate change is threatening ecosystems around the world. Especially the increase in duration, intensity, and frequency of droughts can have a considerable impact on the global carbon cycle. The question whether plants and microbes are susceptible to environmental stress like drought has been assessed in many studies for different ecosystem types and by using numerous approaches, but research on drought effects that includes above- and belowground interactions is rather scarce. Therefore, the present study assesses the question of how drought and/or heat influence the interactions of plants and microbes, especially the carbon coupling, in order to determine the strength of plant-microbe carbon linkages when an ecosystem is pushed to its limits.

The focus of this study thus lies on changes in aboveground-belowground carbon dynamics and the subsequent effects on the soil microbial community under drought and/or heat stress in two climate-threatened ecosystems. It was evaluated how extreme climate events, that are predicted to be more frequent in the near future, affect the carbon coupling between plants and microorganisms and how microbial communities respond under these circumstances, in order to be able to better predict ecosystem resistance and response mechanisms under future climate change.

In chapter 4 a beech forest understory ecosystem was investigated. An extreme climate event (drought and/or heat) was imposed on beech forest monoliths and the strength of the plant-microbe carbon linkages and changes in the microbial community structure and activity were determined by using stable ^{13}C isotope techniques and molecular-based approaches like 16S rRNA and microbial phospholipid-derived fatty acid (PLFA) analysis. In chapter 5 a small aquatic ecosystems was investigated. Two emergent aquatic macrophytes, *Phragmites australis* and *Typha latifolia*, were grown on kettle hole sediment and then exposed to a month-long summer drought in a mesocosm experiment. By conducting a $^{13}CO_2$ pulse labeling as well as PLFA and non-structural carbohydrate analyses, the fate of carbon was traced from the plant leaves to the roots and into the sediment, where some of the recently assimilated carbon is incorporated into microbial PLFAs.

Overall, this study showed that the two investigated ecosystems can endure environmental stress like heat and drought relatively well, at least in the short-term, and that the carbon continuum, or the linkage between above- and belowground communities, remained intact even under severe stress. In conclusion, it seems that ecosystems strongly depend on and try to maintain a functional plant-soil/sediment microorganism carbon continuum under drought, which might help to withstand the increase in extreme drought events under future climate change.

Zusammenfassung

Der Klimawandel bedroht Ökosysteme auf der ganzen Welt. Besonders der Anstieg in Länge, Intensität und Häufigkeit von Dürren kann bedeutenden Einfluss auf den globalen Kohlenstoffkreislauf haben. Die Frage, ob Pflanzen und Mikroorganismen anfällig gegenüber ökologischem Stress wie Dürren sind, wurde bereits in vielen Studien für verschiedene Ökosysteme und mit verschiedenen Ansätzen untersucht, aber Analysen von Dürreauswirkungen, die ober- und unterirdische Interaktionen von Pflanzen und Mikroorganismen mit einbeziehen, sind eher selten. Deshalb wird in der vorliegenden Studie die Frage erörtert, wie Trockenheit und/oder Hitze die Interaktionen von Pflanzen und Mikroorganismen in Bezug auf ihre Kohlenstoff-Verbindung beeinflussen. Dies dient zur Bestimmung der Stärke der Pflanze-Mikroorganismen-Kohlenstoff-Verbindung, wenn das Ökosystem an seine Grenzen gebracht wird.

Der Fokus liegt deshalb auf durch Trockenstress und Hitze hervorgerufenen Veränderungen in der ober-unterirdischen Kohlenstoff-Dynamik in zwei vom Klimawandel bedrohten Ökosystemen. Es wurde untersucht, wie extreme Klimaereignisse, deren Häufigkeit in Zukunft weiter ansteigen soll, die Kohlenstoff-Verbindung zwischen Pflanzen und Mikroorganismen beeinflusst und wie mikrobielle Gemeinschaften unter diesen Umständen reagieren, um die Resistenz und Reaktionsmechanismen von Ökosystemen im zukünftigen Klimawandel besser vorhersagen zu können.

In Kapitel 4 wurde ein Buchenwaldunterholz-Ökosystem untersucht. Buchenwaldmonolithen wurden einem extremen Klimaereignis (Trockenheit und/oder Hitze) ausgesetzt. Die Stärke der Pflanze-Mikroorganismen-Kohlenstoff-Verbindung und Veränderungen in der mikrobiellen Gemeinschaftsstruktur und -aktivität wurden mithilfe von stabilen ^{13}C Isotopenmethoden und Ansätzen auf molekularer Basis, wie 16S rRNA- und Phospholipid-Analysen, bestimmt. In Kapitel 5 wurde ein kleines aquatisches Ökosystems untersucht. Zwei emerse aquatische Makrophyten, *Phragmites australis* und *Typha latifolia*, wurden in einem Mesokosmos-Experiment mit Sediment aus einem Soll einer einmonatigen Dürre ausgesetzt. Mithilfe einer $^{13}CO_2$ Pulsmarkierung, sowie PLFA- und nicht-strukturbildenden Kohlenhydrat-Analysen wurde Kohlenstoff von den Blättern in die Wurzeln bis ins Sediment verfolgt, wo er teilweise in mikrobielle Phospholipide eingebaut wird.

Diese Studie hat gezeigt, dass die zwei untersuchten Ökosysteme Trockenstress und Hitze relativ gut widerstehen können, zumindest kurzfristig, und dass das Kohlenstoff-Kontinuum, beziehungsweise die Verbindung zwischen ober- und unterirdischen Gemeinschaften, auch unter starkem Stress intakt bleibt. Zusammenfassend scheint es, dass Ökosysteme stark von einem funktionierenden Pflanze-Boden/Sediment-Mikroorganismen Kohlenstoff-Kontinuum abhängen und versuchen, es auch unter starkem Stress zu erhalten, was möglicherweise dazu beiträgt, dem Anstieg von extremen Dürreperioden aufgrund des Klimawandels besser zu widerstehen.

1. Introduction

1.1. The symbiosis of plants and microbes – Why we should care!

In a world experiencing dramatic changes in climate, land use, and resource demands, understanding the responses and feedbacks of ecosystems under increasing stress is vital to cope with the challenges lying ahead. However, when looking at ecosystem changes, scientists usually concentrate on certain sections of interest mainly due to the complexity of factors involved. This can lead to simplified or even biased results (Körner 2011; Leuzinger *et al.* 2011) because important ecosystem processes and functions, like plant biomass production and carbon and nitrogen cycling, are greatly influenced through a cascade of biotic and abiotic drivers, direct and indirect effects (see Box 5), and above- and belowground interactions of the plant-soil microorganism continuum. (Steinbeiss *et al.* 2008; Van Der Heijden *et al.* 2008; Brüggemann *et al.* 2011; Schimel & Schaeffer 2012; Bardgett *et al.* 2013).

Plant and soil microbial communities have to deal with a combination of different influencers like climate, physical and chemical soil properties, and the given ecosystem biodiversity. Therefore, they need to adapt to numerous co-occurring environmental conditions to survive (Schimel & Schaeffer 2012). This has led to a strong interdependence between plants and soil microbial communities (Zak *et al.* 2003; Brüggemann *et al.* 2011; Bardgett *et al.* 2013), where plants directly affect microorganisms through the quantity and quality of plant litter and root exudates, and thus indirectly affect the carbon and nitrogen cycle (Zak *et al.* 2003; Fornara & Tilman 2008; Steinbeiss *et al.* 2008; Brüggemann *et al.* 2011; Mellado-Vazquez *et al.* 2016). Microorganisms in turn directly affect plants through symbiotic – a positive relationship enhancing plant productivity and diversity – or pathogenic – a negative relationship reducing plant productivity and diversity – root-associated processes, and thus indirectly affect nutrient availability and resource partitioning (Van Der Heijden *et al.* 2008; Classen *et al.* 2015).

Altering climate conditions due to global climate change are a major concern in terms of future ecosystem functioning because plants and soil are important sinks of carbon, and microorganisms regulate key processes that control carbon and nitrogen cycling. We should be aware that the decoupling of the plant-soil microorganism continuum through environmental stress can, therefore, influence the carbon cycle and disturb the equilibrium of ecosystems (Cox *et al.* 2000; Bardgett *et al.* 2013; Van der Putten *et al.* 2013; Classen *et al.* 2015).

1.2. Plant litter and root exudates – One organisms' trash is another organisms' treasure

Plants and soil microorganisms mainly interact through plant litter and the rhizosphere (see Box 1). Plant litter is an important carbon and nutrient source for decomposers and its quality thus greatly influences soil microbial communities. Plant litter decomposition can be defined as the breakdown of highly organized plant tissue to complex organic compounds that is regulated by both biotic and abiotic processes. Also, since it is a slow process, it can be considered as the bottleneck for a significant portion of primary productivity sent belowground (Brüggemann *et al.* 2011).

Box 1: Rhizosphere

A unique environment located in a narrow zone in the vicinity of roots with different chemical, biological and physical conditions compared to the bulk soil. The rhizosphere arises from the loss of carbon from roots, which is called rhizodeposition, and the consequent proliferation of microorganisms in the surrounding soil, coupled with the physical presence of a root and processes associated with nutrient uptake (Jones et al. 2004).

Another pathway of plant carbon input belowground is carbon allocation (see Box 2) of recent photosynthates to roots and microbial communities, which is a rapid process acting on timescales of hours to days depending on the system (Epron *et al.* 2012; Bahn *et al.* 2013). From leaves – as the source of recently photo-assimilated carbon – assimilates are transported to sink tissues like roots through loading into the phloem (see Box 4) and transport in the sieve tube system (Savage *et al.* 2016). The direction and intensity of the carbon flux is determined by the source-sink relationships, and thus by the source strength, i.e. the extent of provisioning of assimilates, as well as by the ability of the sinks to take up and utilize the available carbon (Lacointe 2000), which is converted to structural compounds, transferred to storage pools, or released into the rhizosphere via rhizodeposition (Lacointe 2000; Brüggemann *et al.* 2011; Gessler & Treydte 2016).

Box 2: Carbon allocation

The flow of carbon to or from an above- and/or belowground component per unit time (= flux) and/or the flux of carbon to a particular component (= partitioning) as well as the carbon distribution in different components (Litton *et al.* 2007).

Rhizodeposition is the input of mainly labile organic compounds with small molecular weight, like sugars, organic and amino acids. The input of these so-called root exudates mostly occurs through uncontrolled passive diffusion because low membrane permeability coefficients and size prevent larger molecules from passing. However, plants have some control over specific purpose-release exudates by opening membrane pores like anion channels which increase the diffusion rate (Jones *et al.* 2004). It has also been shown, that plants produce and release analogs of bacterial signal molecules via root exudates to fight against soil-borne pathogens or to stimulate a specific microbial community composition (Paterson 2003).

Soil microorganisms, on the other hand, can alter rates of soil organic matter (SOM) decomposition through changes in their community composition and can manipulate rhizosphere functioning by producing plant hormones that enhance root growth and with it rhizodeposition (Paterson 2003). Microbes can either increase nutrient availability for plants (positive relationship), e.g., through carbon decomposition or nutrient mineralization or compete for nutrients (negative relationship), especially in strongly nutrient-limited ecosystems (Van Der Heijden *et al.* 2008).

1.3. A mutual relationship – United we stand, divided we fall!

As in every relationship, plants and microorganisms have their differences, for example when they compete for the same limited resources, but in many cases, they benefit from each other because root exudates provide energy for rhizo-dependent microorganisms to mineralize nutrients from SOM (Paterson *et al.* 2007; Classen *et al.* 2015). This, in turn, facilitates plant growth as the mineralized nitrogen is fast available for plants, due to a rapid turnover of microbial cells, which is often referred to as the microbial loop (Paterson 2003; Baptist *et al.* 2015). The so-called rhizosphere priming effect, which can even help plants to drive their own nutrient supply, is the result of accelerated SOM mineralization through increased microbial activity in the presence of plants (Shahzad *et al.* 2015).

In summary, plant growth depends on nutrient availability which is often the limiting factor in soils. Thus, especially in nutrient-poor soils, plants accelerate nutrient cycling through stimulation of microbial transformation of SOM to readily consumable forms by releasing carbon-rich exudates (Paterson 2003). Therefore, microbes are a key factor in regulating and controlling nutrient transformations, plant populations, and nutrient availability and, at the same time, are strongly affected by plants and their activity. If just one part of the plant-soil microorganism continuum is severed, e.g., through changes in biomass allocation patterns and carbon turnover in plants via fertilization (Baptist *et al.* 2015) or drought (Fuchslueger *et al.* 2014), it can have considerable effects on ecosystem processes and functioning (Figure 1-1), including changes in carbon and nitrogen cycling or plant community composition (Classen *et al.* 2015).

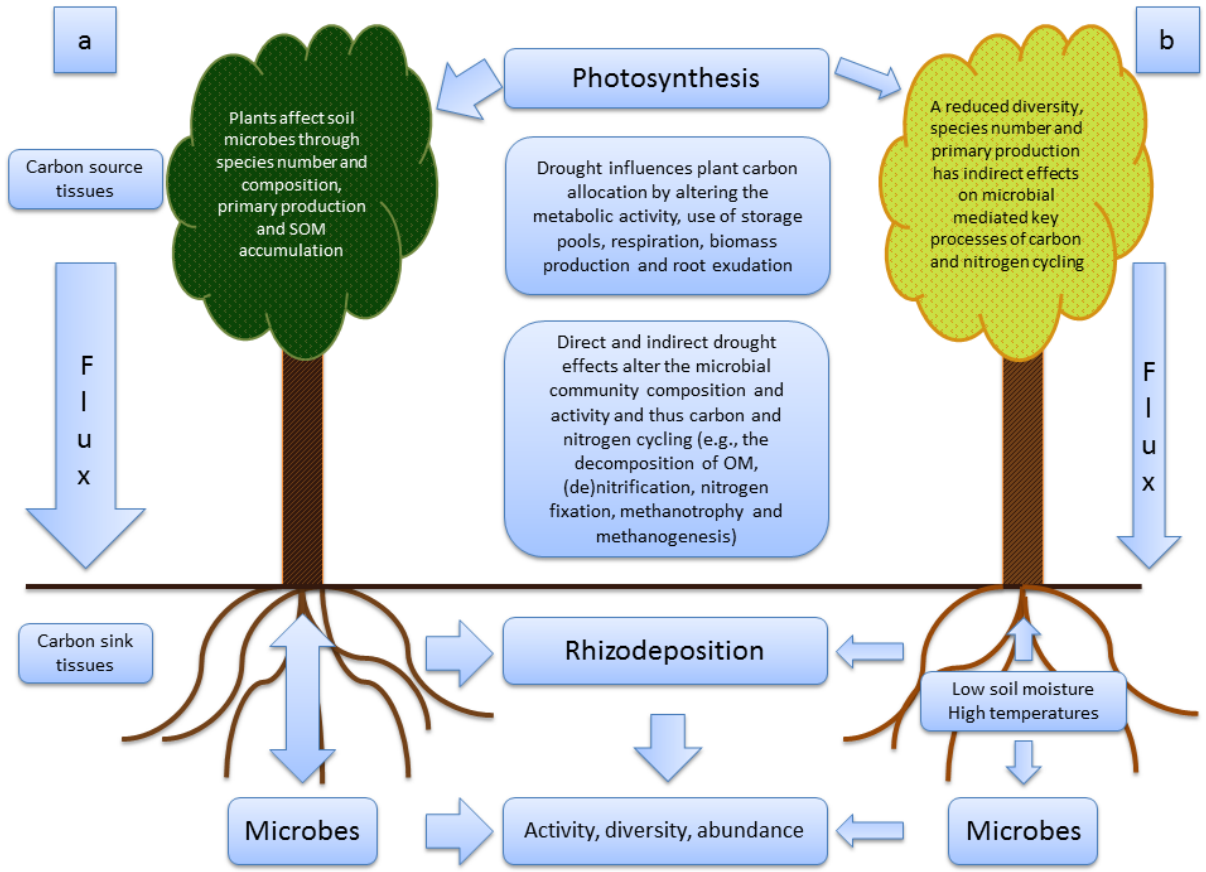

Figure 1-1: Overview of interactions between plants and microbes and the effects on ecosystem nutrient and carbon fluxes under non-stressed (left; a) and stressed (right; b) conditions.

1.4. Climate change – Extremer conditions threaten us all

Ecosystems worldwide face global change (IPCC 2012). In Europe alone, the frequency, duration, and intensity of droughts and heat waves are expected to increase in the near future (Beniston *et al.* 2007; Briffa *et al.* 2009). Causes for droughts are increasing temperatures and changing rainfall patterns due to human-induced rising greenhouse gas (GHG) emissions and the subsequent global warming (Sowerby *et al.* 2005; Dai 2011). The net effect of climate change on ecosystem carbon budgets depends on the balance between photosynthesis and respiration, or in other words, it depends on plants (photosynthesis and autotrophic root respiration) and microorganisms (heterotrophic soil respiration), including the mycorrhiza and associated microorganisms together with roots (mycorrhizosphere respiration or autotrophic soil respiration) (Bardgett *et al.* 2008).

Drought stress is one of the greatest threats to plants and the most widespread factor that influences the carbon balance of forests. Drought can lead to a severe vegetation change and even broad-scale forest die-off events, especially when drought periods are accompanied by heat and appear more frequently and with longer duration (thus gradually depleting even the deeper soil water reserves) (Reichstein *et al.* 2013; Allen *et al.* 2015).

The increase of severe droughts leads to a decrease in soil water availability due to a higher evaporative demand and a precipitation deficit (Briffa *et al.* 2009). Water is the major medium for the transportation of metabolites and nutrients in plants, in addition to being a major component of plant biomass and the central reaction medium for all biochemical processes (Lisar *et al.* 2012). Thus, drought triggers a cascade of morphological, physiological, biochemical, and molecular responses in plants. This includes the decrease of growth rates, photosynthesis, and protein synthesis, as well as morphological changes like reduced leaf size and increased root:shoot ratios. Drought also activates stress response mechanisms, for example, the synthesis of stress response proteins. This includes heat-shock and LEA-type proteins which, among other functions, stabilize and protect macromolecules like enzymes and mRNA from dehydration (Yordanov *et al.* 2000; Sun *et al.* 2002; Wang *et al.* 2004; Lisar *et al.* 2012).

1.5. Starve for survival – The dilemma of plants under drought

To avoid dehydration or hydraulic failure (see Box 3) under drought, plants minimize their water loss by closing their stomata – which regulate CO_2 uptake and transpiration – to reduce the loss of water through the leaves. At the same time, plants maximize their water uptake by increased root growth through adjustment of allocation patterns, at least at the beginning of a severe drought or if the drought is moderate (Chaves *et al.* 2003; Arve *et al.* 2011; McDowell 2011). However, under severe drought root biomass might decrease as a consequence of root mortality and reduced belowground allocation (Hommel *et al.* 2016).

Stomatal closure prolongs the plant survival under drought by maintaining physiological functions and minimizing the reduction of the cell water potential and turgor. However, limited stomatal conductance (gaseous exchange mainly of CO_2 and water) leads to a reduction in growth rate, leaf cooling, and photosynthesis – and thus to an altered uptake and transportation of carbon – and also limits the uptake and transportation of nutrients (Flexas *et al.* 2006; Ruehr *et al.* 2009; Arve *et al.* 2011).

Box 3: Hydraulic failure and carbon starvation

Hydraulic failure occurs when water loss from transpiration is sufficiently greater than uptake by roots, creating high xylem water tensions, and resulting in progressive cavitation and conductivity loss of the xylem (Sevanto *et al.* 2014).	**Carbon starvation is any situation where the carbon supply via photosynthesis, autophagy, and mobilization of non-structural carbohydrates is smaller than the carbon use by respiration, growth, and defense (McDowell 2011).**

Since plant survival not only depends on keeping tissues hydrated but also on carbohydrates that fuel energy demanding processes, plants face the dilemma of reducing transpiration at the expense of carbon assimilation, and both, the water loss and the carbon gain, need to be balanced out carefully to maintain homeostasis (carbon and water balance). One of the key elements in resisting severe drought stress lies in the production and accumulation of osmolytes in cells. Examples of osmolytes in plants are compatible solutes like amino acids (e.g., proline) and water-soluble non-structural carbohydrates (NSCs) like sugars (e.g., sucrose). The accumulation of osmolytes leads to an increase in the osmotic potential, and thus regulates osmotic adjustment and turgor because osmolytes have a high solubility (Lisar *et al.* 2012). It has been observed that water-soluble NSC concentrations in plants increase at the beginning of a drought, when plant growth, and thus the demand for assimilates, declines faster than photosynthesis. With progressing drought, the water-soluble NSC concentrations decline because they are used for maintenance of cellular survival through osmotic adjustment, while, at the same time, the carbon supply via photosynthesis is strongly reduced (McDowell 2011). When carbon concentrations reach critical values, the plants can use autophagy – the breakdown of proteins, lipids, and other materials – which will give a short-term energy source as a last resort (Rose *et al.* 2006). If the remaining water-soluble NSCs are unavailable or fail to meet essential metabolic demands, the plant will succumb to the drought (McDowell 2011).

The mechanisms underlying plant mortality under drought are still poorly understood but it is assumed that plants can either die through hydraulic failure (low plant water potential; see Box 3) or carbon starvation (unavailability of carbon; see Box 3) or, most probably, through a combination of both processes (McDowell 2011; Sevanto *et al.* 2014). Hydraulic failure – as the desiccation from failed water transport – occurs if plants maximize the gas exchange which results in low water potentials, for example under droughts with high intensity. When the loss of water from transpiration is greater than the root uptake it results in high xylem water tension and ultimately leads to a conductivity loss of the xylem (Mitchell *et al.* 2013; Sevanto *et al.* 2014). Carbon starvation is supposed to be a slow process that occurs during droughts with low intensity but long duration, causing prolonged periods without net photosynthesis, where plants regulate gas exchange at the cost of carbohydrate depletion and maintain their water potential until their carbon storage depletes.

Box 4: Xylem and phloem

The xylem is a plant transport system where water and minerals move unidirectionally from roots to other plant organs.	**The phloem is a plant transport system where carbon compounds like sugars and amino acids are transported bidirectional (from source to sink) through the plant.**

However, recently, it is proposed that a coupling of both processes – hydraulic failure and carbon starvation – occurs, where the direct loss of xylem conductivity results in an insufficient water supply of the phloem and thus a reduced phloem transport, because the higher the xylem tension, the harder it is for the phloem to obtain water from it. When the phloem turgor collapses due to a low water potential and sink limitations, a carbohydrate cut off to plant organs occurs – thus reducing NSC use for metabolism and osmoregulation – which ultimately results in hydraulic failure and carbon starvation, even though (non-structural) carbohydrate reserves may not yet be depleted (McDowell 2011; Mitchell *et al.* 2013; Sevanto *et al.* 2014; Savage *et al.* 2016).

1.6. Carbon allocation changes – A consequence of drought

To sustain plant performance and fitness under drier conditions and higher temperatures plants will respond by stomatal regulation and changes in mesophyll conductance and respiration (Flexas *et al.* 2006; Arve *et al.* 2011). Through reduced carbon assimilation and carbon transfer velocity, as well as a potentially longer mean residence time of recent assimilates in leaves, belowground carbon allocation is altered, which ultimately leads to a reduced coupling of above- and belowground processes (Ruehr *et al.* 2009; Brüggemann *et al.* 2011; Reichstein *et al.* 2013; Fuchslueger *et al.* 2014).

Carbon cycling is strongly influenced by carbon allocation through shifting carbon between respiration and biomass production, ephemeral and long-lived tissues, and above- and belowground components (Litton *et al.* 2007). Carbon allocation patterns, in turn, are influenced by resource limitation of water, light, and nutrients and can be controlled by the sink strength, meaning that carbon is allocated to tissues with the highest demand. Thus, as a means to improve the supply of the limited resource, plants will invest in the growth of the responsible uptake organ – e.g., in roots for limited water and nutrients and shoots for limited light – which alters the root:shoot ratio (Lacointe 2000; Bahn *et al.* 2013).

The carbon partitioning, which is controlled by photosynthetic supply of assimilates and the ability of organs to utilize the assimilates, occurs through the loading of assimilates into the phloem (see Box 4), transport in the sieve tube system, and unloading at the sites of demand, thus assimilates are distributed within the plant and between plants and soil (Brüggemann *et al.* 2011). Bahn *et al.* (2013) for example evaluated shading effects (reduced carbon source strength) on belowground carbon allocation in mountain grassland and found that reduced carbon supply led to an increased carbon allocation belowground, at the expense of the aboveground carbon status which may affect the transfer to, and turnover of, newly assimilated plant carbon in soil microbes. They also found that recently assimilated carbon is deposited in shoot starch during the day and remobilized during the night for respiration and growth while root starch rather seems to act as a seasonal store.

When new assimilates are abundant, metabolic activity and growth will mainly be governed by recent assimilates instead of remobilized storage compounds (Gessler & Treydte 2016). The storage of NSCs is a process that allows organisms to buffer resource supply fluctuations, which can be critical for the survival under environmental stress (Palacio *et al.* 2014). Stored and remobilized carbon is used as compensation for sustaining vital plant processes like respiration under extreme heat and drought conditions, which inhibit photosynthesis and thus the supply of new assimilates (Gessler & Treydte 2016). Recent studies also support the idea that under limiting assimilate availability carbon storage is given priority over growth and thus – e.g., under drought stress – growth inhibition is not only due to reduced photosynthesis but also due to a trade-off in carbon utilization (Sala *et al.* 2012; Palacio *et al.* 2014).

1.7. David vs. Goliath – Or how microbes deal with drought

As one of the oldest groups of living organisms on earth, microbes had plenty of time to evolve adaptation and acclimation strategies in order to survive environmental stress like drought. Nevertheless, soil microbial community composition and activity are susceptible to drought (Castro *et al.* 2010; Blankinship *et al.* 2011).

Box 5: Direct and indirect effects of drought

Direct effects of drought occur through the exposure to drought, e.g. via altered precipitation and temperature patterns (Classen et al. 2015).	**Indirect effects of drought are induced by a given set of potential mediators, e.g. changes in species distribution and phenology (Classen et al. 2015).**

Microbes experience drought stress mainly through alterations in microclimate and resources (via reduced substrate diffusion) due to decreasing soil water potentials – so the primary direct stress is physiological and not physical (Schimel *et al.* 2007; Manzoni *et al.* 2012). Direct effects of drought (see Box 5) through, for example, temperature and soil moisture changes affect process rates and inorganic resource availability of microbes which directly influences carbon pools. On the other hand, indirect effects (see Box 5) like changes in species distribution and phenology of plants shift above- and belowground properties – e.g., diversity and community composition – and interactions, and thus indirectly influence carbon pools (Classen *et al.* 2015).

Due to their semipermeable membranes, the intracellular water potential of microbes adjusts quickly to the surrounding soil water potential, which can lead to desiccation during a drought through reduction of the extracellular water potential. In order to avoid dehydration under drought, microbes have to accumulate compatible solutes like proline (Or *et al.* 2007). So, the first stress response of microbes is a resource allocation change from growth to survival pathways, which includes the production of exoenzymes and protective molecules like osmolytes – to reduce water potential and maintain hydration – and chaperones – to stabilize proteins. However, these processes are energetically expensive and strongly decrease the microbial growth rate (Schimel *et al.* 2007; Or *et al.* 2007). Manzoni *et al.* (2012) concluded from a meta-analysis of responses of soil microbial communities to water stress that diffusion limitation in dried soils is probably the main factor limiting microbial activity and thus, longer drought periods in future will likely lead to a slowed nutrient cycling through longer inactivity of decomposers. A last option for microorganisms to flee from unfavorable conditions or even death is to go into a dormant state (Lennon & Jones 2011).

All these adaptations on the small scale lead to large-scale impacts on the environment as microbes are key mediators of carbon and nutrient cycling (Schimel *et al.* 2007). Schimel *et al.* (2007) estimate the amount of carbon consumed for producing osmolytes during a single drought period to 3 to 6% of the total ecosystem annual net primary production (NPP) and for nitrogen to 10 to 40% of annual net nitrogen mineralization in grasslands. How much this influences ecosystem functions depends on the carbon and nitrogen cost of the life strategy of microbes present. In other words, are the microbes resistant (tolerance of stress without implementation of specific mechanisms) or do they have to acclimatize? Inherent resistance comes with a prize, mainly through trade-offs that affect microbial functioning. For example, gram-positive bacteria are probably more adapted to drought than gram-negative bacteria, because of their thicker cell wall, but producing this strong, interlinked peptidoglycan cell wall needs high carbon, nitrogen, and energy inputs (Schimel *et al.* 2007; Fuchslueger *et al.* 2014). On the other hand, acclimation will only lead to a response under stress and the costs of maintaining resistance mechanism genes are relatively small but it requires a redirection of energy and nutrients into survival. It also proposes the risks of the unavailability of needed resources or the stress being too rapid to acclimate (Schimel *et al.* 2007).

1.8. The importance of microbes – Why size does not always matter (part 1)

Microbial decomposers play a fundamental role in the decomposition process in soils as they regulate the rate-limiting steps. Therefore, they also regulate the influence of abiotic factors on decomposition. For example, the soil will become a source of carbon (carbon flux from the soil to the atmosphere) if the decomposition rate increases relative to inputs coming from plants and animals (Classen *et al.* 2015). With decreasing soil moisture, solute and enzyme mobility – and thus the

substrate supply for microbes – is reduced and the metabolic activity of most soil microbes decreases. In addition, the decomposition rate of plant litter decreases because decomposers that are more tolerant to drought, for example, gram-positive bacteria and fungi, seem to have a lower metabolic capacity. This leads to a decline in respiration and nutrient mineralization (Schimel *et al.* 2007; Manzoni *et al.* 2012; Fuchslueger *et al.* 2014). Therefore, the soil microbial community – next to factors like climate, topography and vegetation type – regulates the amount of carbon that can be stored in or released from soils. The annual flux of carbon into and out of terrestrial ecosystems is huge (around 120 Gt) and thus small changes in the carbon and nitrogen allocation can have a high impact on the terrestrial carbon cycle, leading to an imbalance in natural ecosystems themselves (Classen *et al.* 2015). However, the responses of microorganisms (or of soil respiration) to drought are still poorly understood. The difficulty of predicting microbial responses lies in the numerous and complex interactions and feedbacks of the soil microbial community with factors like climate and plants (Bardgett *et al.* 2008). Thus, for a substantial and significant forecast of the interactions between the global carbon balance and the future climate, and the subsequent environmental changes, it is inevitable to study and understand drought responses of the plant-soil microorganisms continuum in terrestrial as well as aquatic ecosystems.

1.9. The importance of forests – Impacts of stressed forests on the carbon cycle

Forests are susceptible and highly sensitive to drought because of their large carbon pools and fluxes and their long biomass recovery time, which leads to an immediate as well as lagged and thus potentially long-lasting influence on the carbon balance (Reichstein *et al.* 2013; Frank *et al.* 2015). Ciais *et al.* (2005), for example, estimated a 30% reduction in gross primary productivity of forests over Europe due to the strong drought and heat wave event in 2003, which reversed the effect of four years of net ecosystem carbon sequestration. Therefore, Frank *et al.* (2015) even assume that forests “exhibit the largest net effect of extremes” and that droughts “have the strongest and most widespread effects on terrestrial carbon cycling”.

Forests store around 45% of the carbon found in terrestrial ecosystems (Anderegg *et al.* 2012; Reichstein *et al.* 2013) but climate change can reduce their uptake by up to 54% (Friedlingstein *et al.* 2001). Extreme climate events like droughts disturb forest ecosystems to the extent of carbon stock net losses through changes in carbon sink strength, due to immediate or time-lagged responses such as community changes, reduced activity, or mortality (Reichstein *et al.* 2013; Anderegg *et al.* 2016). Therefore, it is highly important to estimate forest vulnerability and response mechanisms to drought in order to understand climate cycle feedbacks due to changing carbon source and sink strengths and with it the carbon cycle under a changing world climate.

1.10. The importance of small water bodies – Why size does not always matter (part 2)

When looking at the terrestrial carbon cycle small continental freshwater ecosystems have largely been ignored in terms of regional and global influence on the carbon cycle due to their small surface area. They were usually treated as insignificant or were only seen as reservoirs that hold water and materials for a short time without much processing (Cole *et al.* 2007; Downing 2010). However, small freshwater ecosystems like kettle holes (see Box 7) are vital elements for the carbon balance and play a major role in global cycles because they are more active in almost every biogeochemical process when compared to large lakes, terrestrial, and marine ecosystems (Downing 2010).

Box 7: Kettle holes

Kettle holes are glacially created, small, shallow, depressional water bodies that collect their water from internal or closed catchments in young moraine landscapes. Their water body is defined by having a maximum extent of 1 ha (Kalettka & Rudat 2006).

Ponds and kettle holes have been underestimated in the past in terms of abundance and importance for aquatic biodiversity and global carbon cycles (Downing *et al.* 2006; Boix *et al.* 2012; Pätzig *et al.* 2012). However, new assessments suggest that the global extent of natural lakes is twice as large as previously known, covering more than 3 % of the earth's surface, and thus, small water bodies and not large ones seem to represent the most lacustrine area (Downing *et al.* 2006). For example in Northeast Germany, thousands of kettle holes are scattered over the agricultural landscape and cover up to 5% of the arable land (Kalettka *et al.* 2001). They also function as biodiversity hotspots that are important for macrophyte species richness and are refuges for endangered species (Pätzig *et al.* 2012). Kettle holes can often be found on agricultural fields in young moraine landscapes. With this, they can be considered small islands of biodiversity in intensively used farmlands and thus provide shelter for a multitude of organisms. Due to the lack of an integrated drainage network kettle holes are closed flow systems and undergo severe wet-dry cycles with extremely variable water dynamics (Kalettka *et al.* 2001; Kalettka & Rudat 2006; Gołdyn *et al.* 2015). Downing (2010) concluded that "the large area covered by small aquatic systems and the intensity of activity mean that they may be among the most important ecosystems in the world". Thus, a deeper understanding of the functioning of inland freshwater ecosystems like kettle holes under future climate is necessary.

2. Introduction of Methods

2.1. Isotopic tracers – Assessing the fate of assimilates in an ecosystem

Box 8: ^{13}C isotopes and labeling

^{13}C is a stable isotope of carbon. Isotopes have the same number of protons in each atom but differ in neutron number so they have different atomic weights. ^{12}C forms almost 99% of the Earths carbon, ^{13}C only around 1%. Thus, by supplying the plant with $^{13}CO_2$ (labeling) it is possible to use ^{13}C as a tracer for the fate of recently assimilated carbon.

Labeling approaches are widely used to investigate carbon allocation, sequestration, and turnover, or in short, the fate of carbon in the plant-soil microorganism-continuum. Basically, the isotope content of assimilated carbon is artificially altered by labeling plants with ^{13}C enriched $^{13}CO_2$ (Figure 2-1). Since ^{13}C only accounts for about 1.1% of all natural carbon on Earth it can be used as a tracer. Thus, with the rapid transport of recent photosynthates belowground and the release to the soil via root exudates, the transfer and fate of the recently assimilated carbon within the plant-soil system can be traced and quantified (Högberg *et al.* 2008; Ruehr *et al.* 2009; Epron *et al.* 2012).

Early labeling studies date back to the 1950s, for example, by studying the fate of carbon in photosynthetic products in leaves. Also, next to ^{13}C other isotopic tracers exist, including ^{11}C, ^{14}C, and ^{18}O (Epron *et al.* 2012). By labeling plants with ^{13}C and tracing its way from entering the leaves, being transported to roots and the rhizosphere, then being released into the soil through rhizodeposition, and finally being decomposed by soil microorganisms and integrated into their metabolism, it is possible to study the relationship between carbon fixation and its delivery to a defined sink like the soil microorganisms (Klumpp *et al.* 2007). The labeling of microbial biomarker molecules such as DNA, RNA, and phospholipid fatty acids (PLFAs) can help to identify the microorganisms that metabolize the recently assimilated plant-derived carbon and links differences in microbial community structure and activity to environmental changes and plant functioning (Denef *et al.* 2009).

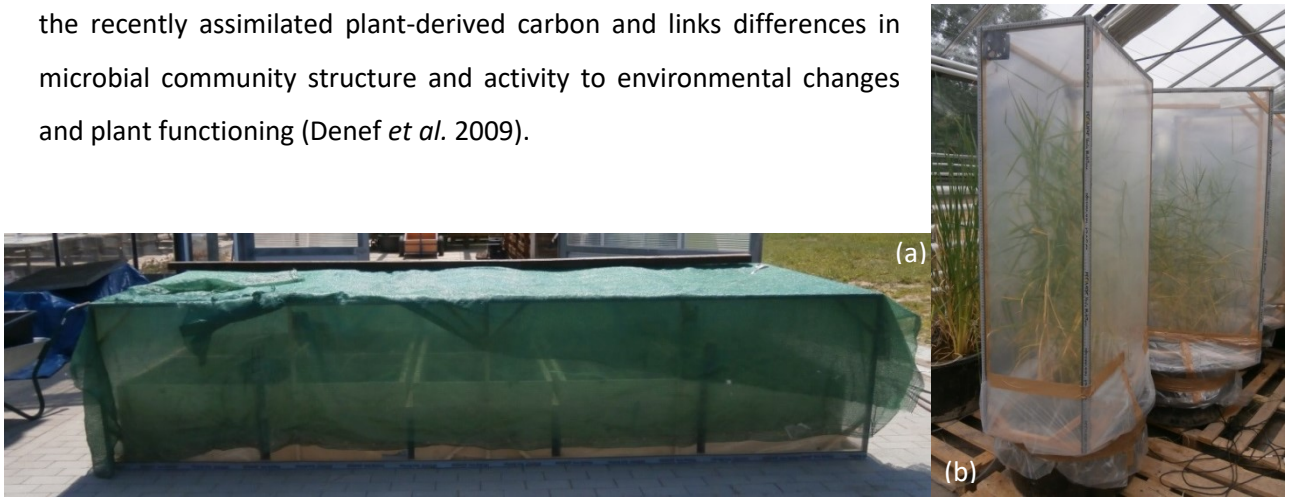

Figure 2-1: $^{13}CO_2$ pulse labeling of (a) the beech forest understory monoliths and (b) *P. australis*

2.2. The microbial community – How to detect the unseen?

It is very difficult to completely determine microbial communities in soil due to their immense phenotypic and genetic diversity. Not only are soil microbes abundant and highly diverse – estimates suggest up to 10^9 cells per gram soil – but they are also hard to cultivate with currently only around 1% of bacterial populations being cultivable (Kirk *et al.* 2004; Gans *et al.* 2005; Armougom & Raoult 2009). Also, when studying microbial diversity and community structure we have to deal with problems like the heterogeneity of soil and the spatial distribution of microbes which lead to biased results in favor of dominant species (Kirk *et al.* 2004). Thus, when studying soil systems in the past, the microbial community was regarded as a "black-box" (Frostegård *et al.* 2011). However, the growing need of opening the black-box to further understand soil processes and ecosystem interactions led to the rise of molecular-based – e.g., DNA and RNA analysis – and biochemical-based methods – e.g., phospholipid-derived fatty acid analysis (Kirk *et al.* 2004; Frostegård *et al.* 2011).

2.3. Molecular-based approaches – 16S rRNA analysis

When molecular-based models started to gain popularity it was like opening a door to a whole new world. Woese (1987) remarked in his famous review that: "The cell is basically a historical document, and gaining the capacity to read it (by the sequencing of genes) cannot but drastically alter the way we look at all of biology. No discipline within biology will be more changed by this revolution than microbiology, for until the advent of molecular sequencing, bacterial evolution was not a subject that could be approached experimentally." By using genotypic information through sequencing of proteins and nucleic acids, a more reliable, precise, and informative interpretation of evolutionary relationships, especially when compared to phenotypic information, can be achieved (Woese 1987).

In order to fulfill the requirements of a good phylogenetic marker, a molecule has to have certain characteristics like ubiquitous occurrence in all organisms, random sequence changes, a great phylogenetic range, a high degree of functional constancy, and a large enough size to ensure sufficient information (Woese 1987; Glaeser & Kämpfer 2015). One type of molecule that fits all the needed characteristics and in addition can be rapidly sequenced is the ribosomal RNA (rRNA). Thus, the most widespread marker gene today for basic evolutionary analysis of both cultivable and uncultivable bacteria is the small subunit (SSU or 16S) rRNA gene that enables the identification and comparison of bacterial phylogeny and taxonomy through phylogenetic analyses (Glaeser & Kämpfer 2015; Singer *et al.* 2016).

Also, RNA-based methods are more suitable for the analysis of metabolically active microbes, for example, when compared to DNA-analyses, since DNA can exist in dead cells and as extracellular DNA and many soil microorganisms are in an inactive state (Felsmann *et al.* 2015). However, even though 16S rRNA analysis is the basic approach for evaluating overall phylogenetic relationships, it sometimes provides an insufficient resolution at the genus and species level. A method that is more efficient at the genera and species level is the so-called multilocus sequence analysis (MLSA), which uses protein-coding genes – that evolve at a slow but constant rate and thus have a better resolution power – as genetic markers (Glaeser & Kämpfer 2015).

Recent advances in sequencing technologies led to reduced costs and labor times and increased data production and thus launched an era of the so-called next-generation sequencing (NGS) which even enables analysis of the entire microbial community within a sample with cultivation being unnecessary. Therefore, instead of dealing with labor-intensive experiments we now face huge amounts of data.

16S rRNA gene high-throughput sequencing (HTS) is a widely used method for microbial diversity evaluation (Mardis 2008; Ju & Zhang 2015). The extraction of RNA from soil is complicated but can be summarized in three stages: cell lysis (e.g., through bead beating), extraction of RNA from the soil matrix (e.g., through phenol extraction), and purification of RNA (e.g., through spin columns). Then the rRNA has to be transcribed into cDNA which can be used as genomic DNA in all subsequent DNA-based approaches, for example, for Polymerase chain reaction (PCR)-based sequencing methods (Wang *et al.* 2012). After sequencing the 16S rRNA, three steps are necessary to analyze HTS data: (1) pretreatment of raw sequence data, (2) microbial diversity analysis, and (3) advanced data analysis and visualization. Programs like 16other help to trim, filter, analyze, and visualize large amplicon sequence data from the NGS (Ju & Zhang 2015).

The database development, or the collection and classification of sequences, was and still is difficult, especially for environmental 16S rRNA gene sequences, due to a high number of novel taxa without cultivated representatives. Thus, correct analysis of data is challenging because of reference sequences with low read accuracy, chimeric sequences, and partial rRNA gene sequences. Also, results vary through primer choice, read length, environmental source, reference database, and assignment, which can influence the estimation of taxon abundance (Singer *et al.* 2016). Nevertheless, precise recommendations for sequence lengths, alignment procedures, and a comparison with well-managed databases now usually lead to good phylogenetic analyses based on 16S rRNA gene sequences (Glaeser & Kämpfer 2015).

2.4. Biochemical-based approaches – Phospholipid fatty acid analysis

Box 10: Phospholipid-derived fatty acids (PLFAs)

Fatty acids are the key component of the cellular membrane of all living cells. Phospholipids consist of a hydrophilic head – including a polar head group and a glycerol backbone – and a hydrophobic chain – the fatty acid chain of saturated and unsaturated fatty acids. Thus, these lipids are asymmetric, having hydrophilic and hydrophobic regions. In the membrane, they form a bilayer with hydrophilic ends towards the outer surface of the membrane and hydrophobic ends buried in the interior (Kaur et al. 2005).

Phospholipid fatty acids (PLFAs) are important components of cellular membranes (Figure 2-2 and Box 10). The so-called signature fatty acids (biomarkers) are used to differentiate major groups within a microbial community, due to differences in the relative abundance of certain PLFAs – e.g., i16:0 is a biomarker for gram-positive bacteria. Differences in the PLFA profile in different treatments are therefore representative of changes in the soil microbial community structure (Kirk *et al.* 2004; Kaur *et al.* 2005; Gómez-brandón & Domínguez 2010).

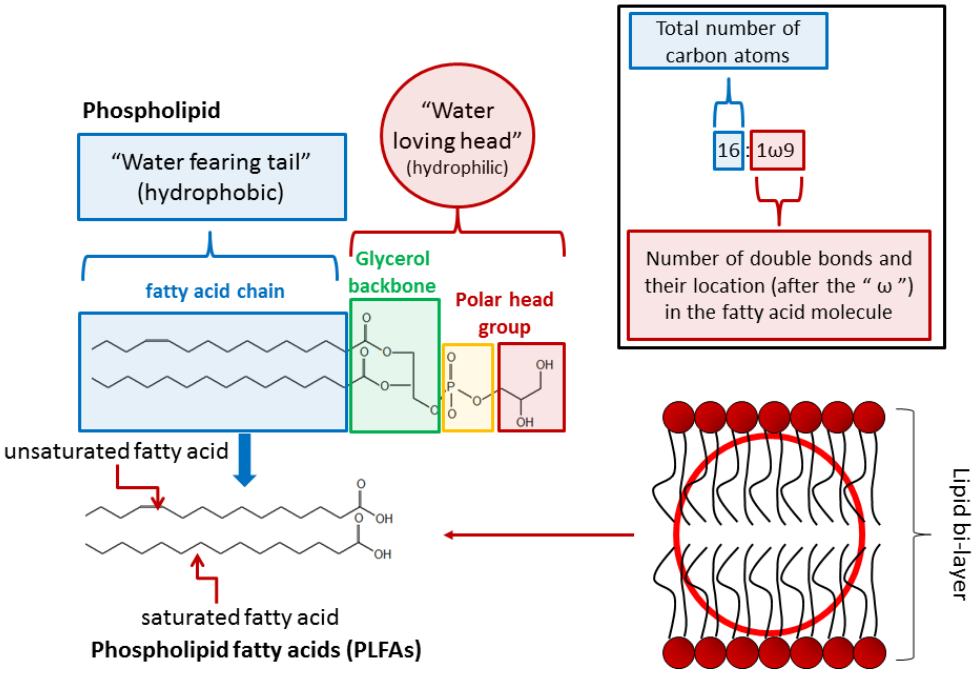

Figure 2-2: Structure of phospholipid fatty acids (PLFAs) and an example of PLFA nomenclature in the black box (prefixes "Me," "cy," "i," and "a" stand for the methyl group, cyclopropane group, and iso- and anteiso-branched fatty acids, respectively).

Using PLFAs as markers has its origins in the 1980s with pioneering work from David White and first experiments in soil systems from Tunlid *et al.* (1989) (Frostegård *et al.* 2011). For analysis, PLFAs are esterified to form fatty acid methyl esters (Figure 2-3) which are then analyzed using gas chromatography – mass spectrometry to obtain a "fingerprint" of the microorganisms in the sample (Gómez-brandón & Domínguez 2010). This can provide information on microbial biomass and community composition of soil and sediment samples, and when combined with ^{13}C stable isotope pulse labeling techniques can even identify active microbial groups that, for example, consume rhizodeposited carbon which provides information on nutritional status and metabolic activity (Treonis *et al.* 2004; Denef *et al.* 2009; Frostegård *et al.* 2011; Buyer & Sasser 2012). Also, with ^{13}C labeled substrates like glucose, it becomes possible to analyze metabolic functions without cultivation through the incorporation of ^{13}C into specific PLFAs (Rinnan & Bååth 2009). Thus, through the combination of PLFA and stable isotope analysis, there is a currently unique opportunity to determine carbon sources of bacteria and fungi.

Problems of PLFA analyses occur via changes in the PLFA composition through external factors like temperature and the possible alteration of FAME profiles through other organisms. In addition, the specificity of PLFAs is rather low. This means PLFAs cannot be used to represent a certain species (no information on species composition) because individuals can have numerous fatty acids and the same fatty acid can occur in more than one species (Kirk *et al.* 2004; Kaur *et al.* 2005; Middelburg 2014). One of the biggest advantages of PLFA methods, when compared to other microbial community analyses, is that it reflects the currently active community, because PLFAs are rapidly synthesized during microbial growth, are not found in storage molecules, and degrade rapidly during cell death (Kaur *et al.* 2005; Gómez-brandón & Domínguez 2010; Frostegård *et al.* 2011; Middelburg 2014). Also, in contrast to nucleic acid based methods like rRNA analyses, PLFA analyses are rapid, inexpensive, provide a broad diversity measurement at the phenotypic level, and could even be more sensitive when looking at shifts in the microbial community composition because molecular analyses reveal the total diversity which is so great that stress response detection on the fine scale is difficult. For a list of used PLFAs, their nomenclature, and extraction methods see e.g., Kaur *et al.* (2005).

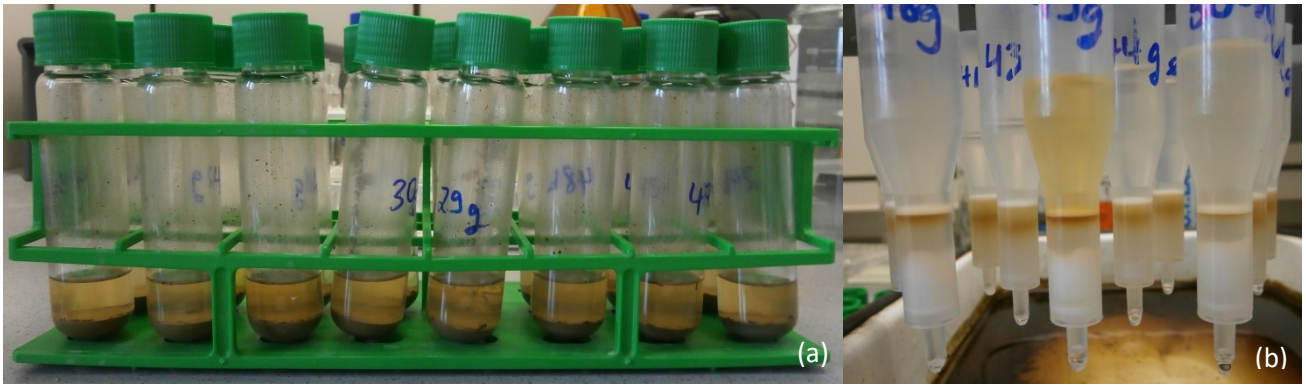

Figure 2-3: PLFA extraction: (a) Extraction of lipids from the sediment with a one-phase Bligh/Dyer solution, (b) Lipid fractionation by solid phase extraction with silicic acid columns

3. About this study

3.1. Overview

With the projected increase in drought duration and intensity in future, drought stress effects on the plant-soil microorganism carbon continuum may disrupt the tight linkage between plants and microbes, which governs soil carbon and nutrient cycling. The present study aims to connect information from above- and belowground processes, spanning from plants to the soil and sediment microbial communities under drought and/or heat stress in a forest (Chapter 4) and aquatic ecosystem (Chapter 5) by using a combination of isotopic, molecular, and biochemical-based approaches (Table 3-1).

Table 3-1: Summary of the conducted experiments and analyses in this study.

Chapter	Ecosystem	Environmental stress	Methods used
4	Forest understory terrestrial *Fagus sylvatica* L.	Heat and/or drought	$^{13}CO_2$ pulse labeling ^{13}C analysis in plant organic matter ^{13}C-PLFA analysis 16S rRNA analysis
5	Kettle holes aquatic *P. australis* *T. latifolia*	Drought	$^{13}CO_2$ pulse labeling ^{13}C analysis in plant organic matter ^{13}C-PLFA analysis NSC analysis

3.2. Objectives and hypotheses

The overall objective of this study is to assess plant-microbe interactions – especially in terms of carbon coupling – in different ecosystems and how they differently react to climate-driven stress, in order to understand what regulates the strength of plant-microbe linkages under different environmental stressors. This will help to evaluate how ecosystems react to environmental change, if and how different plants, microbes, and ecosystems cope with drought stress and to elucidate the underlying response and resistance mechanisms.

Objective 1: Determining the strength of plant-microbe carbon linkages and characterizing changes in the soil-microbial community under drought and/or heat stress in a forest ecosystem.

Chapter 4 contains a short-term study in a semi-controlled environment with the focus on beech forest understory as an example of a terrestrial ecosystem that is threatened by the projected increase in drought duration and intensity in future. In order to assess above- to belowground interactions of plants and microbes under environmental stress, an extreme climate event was imposed on forest monoliths to determine the strength of the plant-microbe carbon linkages, changes in the microbial community, and changes in characterized microbial groups that are tightly linked to the plant-soil carbon continuum. The monoliths with an intact understory plant-microorganism community were excavated from a beech forest (*Fagus sylvatica* L.) in Germany and were imposed with a month-long drought and/or a single heat-pulse event. The understory vegetation was labeled with $^{13}CO_2$ after 2 weeks of treatment to characterize the carbon transport dynamics and to study the relative arrival events of labeled assimilates to belowground plant tissues, microbes, and microbial PLFAs. Using ^{13}C-labeled PLFAs and high-throughput sequencing of the 16S rRNA of bacteria the metabolically active soil microorganisms were characterized and short-term changes in the community structure of metabolically active bacteria upon the stress treatments and the related changes in plant assimilate transfer belowground were determined.

My hypotheses were as follows:

(1) The extreme temperature and heat treatments will result in the plant-soil microbial community linkage to be severed.

(2) The drought and heat-stressed treatments will lead to a lower ^{13}C label in the PLFAs relative to those from the well-watered control and ^{13}C label in the microbial community will be near absent when the carbon continuum is completely severed.

(3) If the treatments result in primarily indirect climate effects (e.g., changes in plant carbon allocation belowground) then we will observe a shift in the bacterial community that directly corresponds to the treatment effect on the understory vegetation.

Objective 2: Determining the strength of plant-microbe carbon linkages and characterizing changes in the sediment microbial community under drought stress in an aquatic ecosystem (kettle holes).

Chapter 5 contains a short-term mesocosm experiment with the focus on kettle holes as an example of aquatic ecosystems that are threatened by the projected increase in drought duration and intensity in future. Since kettle holes are small water bodies, the terrestrial-aquatic interfaces will be subjected to longer dry periods with desiccation of the sediment, which leads to potential negative impacts on the carbon sequestration of small freshwater ecosystems (Reverey *et al.* 2016). Therefore, the fate of carbon from leaves to sediment microbial communities was evaluated through a $^{13}CO_2$ pulse labeling approach, using two emergent aquatic macrophytes – *Phragmites australis* and *Typha latifolia* – as well as sediment taken from a kettle hole. To assess above-to-belowground interactions, the drought response and the plant-sediment microorganism carbon coupling of the two cosmopolitan wetland species was investigated. This includes the characterization of the partitioning of recent ^{13}C-labeled assimilates to different plant compartments (leaves, stems, and roots) as well as the transport to rhizo-dependent microorganisms through the incorporation of ^{13}C into microbial PLFAs.

My hypotheses were as follows:

(1) Drought impairs the carbon transport within plants, causing a reduction in the amount of carbon allocated belowground and a reduction in carbon transport velocities.

(2) Carbon partitioning is driven by osmotic adjustment as well as by the reduction of belowground sink activity due to a reduced demand of roots through impaired growth and metabolic activity.

(3) Drought reduces the ^{13}C label incorporation into microbial PLFAs, with drought effects on the microorganism carbon metabolism potentially being both direct and indirect through reduced carbon delivery from the plant.

In chapter 6 I will first summarize the results of my two studied ecosystems – a beech forest understory and an aquatic kettle hole – in terms of changes in the plant-soil/sediment carbon continuum under environmental stress. Then I will compare both ecosystems in terms of drought reaction and resistance mechanisms of above- and belowground communities and their interactions.

A collective list of the participating co-authors and their affiliations of the manuscripts presented in this thesis (List of co-authors) as well as all figures and tables from the appendices (Appendix) can be found at the end of this thesis. All references cited in the manuscripts were combined to one list (References).

4. Plant-microbe interactions under drought in a forest understory

Forest understory plant and soil microbial response to an experimentally induced drought and heat-pulse event: the importance of maintaining the continuum.

This manuscript was published in the international peer-reviewed journal Global Change Biology. The original article was published by John Wiley & Sons Inc.:

von Rein I., Gessler A., Premke K., Keitel C., Ulrich A., Kayler Z.E. (2016) Forest understory plant and soil microbial response to an experimentally induced drought and heat-pulse event: the importance of maintaining the continuum. Global Change Biology 22:2861–2874.

4.1. Abstract

Drought duration and intensity are expected to increase with global climate change. How changes in water availability and temperature affect the combined plant-soil microorganism response remains uncertain. We excavated soil monoliths from a beech (*Fagus sylvatica* L.) forest, thus keeping the understory plant-microbe communities intact, imposed an extreme climate event, consisting of drought and/or a single heat-pulse event, and followed microbial community dynamics over a time period of 28 days. During the treatment, we labeled the canopy with $^{13}CO_2$ with the goal of (i) determining the strength of plant-microbe carbon linkages under control, drought, heat, and heat-drought treatments, and (ii) characterizing microbial groups that are tightly linked to the plant-soil carbon continuum based on ^{13}C-labeled PLFAs. Additionally, we used 16S rRNA sequencing of bacteria from the Ah horizon to determine the short-term changes in the active microbial community. The treatments did not sever within-plant transport over the experiment, and carbon sinks belowground were still active. Based on the relative distribution of labeled carbon to roots and microbial PLFAs, we determined that soil microbes appear to have a stronger carbon sink strength during environmental stress. High-throughput sequencing of the 16S rRNA revealed multiple trajectories in microbial community shifts within the different treatments. Heat in combination with drought had a clear negative effect on microbial diversity and resulted in a distinct shift in the microbial community structure that also corresponded to the lowest level of label found in the PLFAs. Hence, the strongest changes in microbial abundances occurred in the heat-drought treatment where plants were most severely affected. Our study suggests that many of the shifts in the microbial communities that we might expect from extreme environmental stress will result from the plant-soil microbial dynamics rather than from direct effects of drought and heat on soil microbes alone.

4.2. Introduction

Current and impending climate change is predicted to result in modified temperature and precipitation regimes causing potentially severe alterations of ecosystem functioning, biogeochemistry, and community patterns (IPCC 2012; Reichstein *et al.* 2013; Bahn *et al.* 2014). In Europe, the frequency, duration, and intensity of droughts and heat waves are expected to increase (Schar *et al.* 2004; Beniston *et al.* 2007; Briffa *et al.* 2009; Fischer & Schar 2010) and evidence of climate change impacts on important ecosystem properties, functions and services is emerging. These include shifts in phenology, animal and plant species' distribution (Walther *et al.* 2002), and primary productivity (Ciais *et al.* 2005). But other responses, such as microbial community shifts, are not readily apparent, ostensibly due to a high level of microbial phenotypic plasticity (Merilä & Hendry 2014), functional redundancy within soil communities (Lennon *et al.* 2012; Griffiths & Philippot 2013), and distinctive resistance and resilience of soil microorganisms (Shade *et al.* 2012; Griffiths & Philippot 2013).

Species, communities, and ecosystems have revealed a strong tolerance or resistance to a wide range of environmental variation (Scheffer & Carpenter 2003; Lennon *et al.* 2012; Placella *et al.* 2012; Manzoni *et al.* 2014). Accordingly, not all experiments designed to simulate climate change have resulted in a corresponding response from the community or ecosystem (Smith 2011; Hoover *et al.* 2014), which is why ecological researchers have recently focused on experiments that induce extreme climate events with the goal of identifying critical thresholds and their underlying mechanisms (Reichstein *et al.* 2013; Kayler *et al.* 2015). Smith (2011) defined a climate extreme as a statistically rare event that can "alter ecosystem structure and/or function well outside the bounds of what is considered typical or normal variability". Research based on extreme events has already yielded insights into belowground dynamics (Evans & Wallenstein 2014), but these experiments have largely been carried out using laboratory soil incubations (Barcenas-Moreno *et al.* 2009; Riah-Anglet *et al.* 2015), thus separating linkages between vegetation and soil microbes. Important questions remain about the relevance of the plant-soil microorganism carbon continuum in extreme climate event scenarios, including at which point (i.e., threshold) is the plant-soil microorganism connectivity lost? And, how will microbial communities respond when pushed to their niche limits?

Plants influence microbial communities and functions in multiple ways. Plant effects include the amelioration of the environment, such as soil temperature and moisture (Waldrop & Firestone 2006), physiological and life strategies of plants that influence litter quality (Hobbie 1992; Aerts & Chapin III 1999; Prescott & Grayston 2013), and carbon allocation patterns (Litton *et al.* 2007). Soil microbial community function, on the other hand, can regulate plant diversity-productivity patterns (Van Der Heijden *et al.* 2008; Schnitzer *et al.* 2010; Schnitzer & Klironomos 2011), nutrient availability, and cycling (Bonkowski & Roy 2005; Wagg *et al.* 2014), and may even boost plant fitness to

environmental stress or affect their evolution (Lau & Lennon 2011). Whether top-down or bottom-up control is at play, important ecosystem functions result from the plant-soil microorganism continuum (Bardgett *et al.* 2005; Gilliam *et al.* 2014), which is often severed due to changes in temperature and precipitation regimes (Evans & Wallenstein 2014).

Drought and heat stress can impact soil microorganisms through both direct (e.g., modification of soil structure and pore connectivity in soils) and indirect effects (e.g., reduction in plant net primary productivity resulting in lower microbial carbon availability) (Bardgett *et al.* 2008). Drought has a strong influence on carbon assimilation in plants, affecting stomatal and mesophyll conductance (Hommel *et al.* 2014), leaf biochemistry, and hydraulic pathways (Flexas *et al.* 2006; Resco *et al.* 2009), as well as phloem loading which can result in a reduction in the carbon transfer from the plant canopy to the roots and to soil microorganisms (Ruehr *et al.* 2009). Additional to the reduction in carbon input from plants into the soil (an indirect effect of climate change), mass transfer of reduced substrates within the soil (e.g., dissolved organic carbon) to microbial communities slows (a direct effect of climate change) due to diminished pore connectivity in dry soil (Schimel & Schaeffer 2012; Manzoni *et al.* 2014). The reduction of soil moisture also limits the ability of microbes to migrate to available substrates (Manzoni et al., 2014), or can alter the chemistry of the soil (e.g., acidification) affecting carbon turnover (Clark *et al.* 2005). A soil water potential of -14 MPa, far below the permanent wilting point for plants, has been suggested as the level at which substrate availability to microorganisms is limited by mass transfer (Manzoni *et al.* 2012). With linkages to plants severed resulting in a reduced supply of plant-derived assimilates, microbes can alter their physiology (Csonka 1989; Allison *et al.* 2010; Crowther *et al.* 2014) and/or change their carbon allocation (Schimel & Schaeffer 2012), for example, by producing extracellular enzymes or accumulating osmolytes to maintain cell integrity (Csonka 1989; Schimel *et al.* 2007).

Observations and syntheses of microbial community response to climate change, including drying and warming, are emerging; however, resolving stress-response strategies of microorganisms remains an ongoing challenge in environmental microbiology (Schimel *et al.* 2007; Lennon *et al.* 2012; Evans & Wallenstein 2014). For example, fungi have been shown to have a high tolerance for water stress, often attributed to their ability to spatially explore the soil better for water and nutrients (Frey *et al.* 2008; Riah-Anglet *et al.* 2015). Additionally, due to their differences in cell wall structure, fungi and gram-positive bacteria (which have a thick, interlinked peptidoglycan cell wall), are considered to have wide niche breadths with respect to soil moisture ranges and a stronger tolerance to desiccation (Schimel *et al.* 2007; Lennon *et al.* 2012). Yet, given the multiple and often conflicting community changes observed with modern sequencing tools, generalizations remain elusive, although it is interesting that ecological strategies appear to be grouped at a coarse taxonomic level (phylum) (Lennon *et al.* 2012).

To understand how the plant-soil microorganism continuum responds to impending climate change, including climate extremes, we need to maintain the plant-soil carbon continuum and push the plant and microbial communities beyond their current evolutionary niche boundaries (Bahn *et al.* 2014; Kayler *et al.* 2015). We excavated monoliths from a beech (*Fagus sylvatica* L.) forest in Germany, thus keeping the understory plant-microorganism communities intact, and imposed an extreme climate event, consisting of drought and/or a single heat-pulse event. During the treatment, we labeled the understory vegetation with $^{13}CO_2$ and then followed microbial community dynamics over a short time period of 28 days. Our overarching goal was to understand how the forest understory may react to future climate change, by balancing the simplicity of a short-term extreme climate event in a semi-controlled environment with the complexity of the plant-soil microorganism system response, focusing on plant-microorganism linkages and changes in microbial community structure. Specific aims and hypotheses of the study were as follows:

1. Characterize the carbon transport dynamics by studying the relative arrival events of labeled assimilates to belowground plant tissues and microbial phospholipid-derived fatty acids (PLFAs), allowing the assessment of the strength of plant-microbe linkages under the different treatments. Based on the label patterns, we hypothesize (i) that the extreme temperature and heat treatments will result in the plant-soil microbial community linkage to be severed.

2. Characterize metabolically active soil microorganisms using ^{13}C-labeled isotopic PLFAs that are tightly linked to the plant-soil carbon continuum as the environmental stress increases. Implicit to the canopy labeling is the hypothesis (ii) that the drought and heat stressed treatments will lead to a lower ^{13}C label in the PLFAs relative to those from the well-watered control and will be near absent when the carbon continuum is completely severed.

3. Determine the short-term changes in the community structure of the metabolically active bacteria to the stress treatments and the related changes in plant assimilate transfer belowground, using high-throughput sequencing of the 16S rRNA of bacteria from the Ah horizon of the soil. We hypothesize (iii) that if the treatments result in primarily indirect climate effects then we will observe a shift in the bacterial community that directly corresponds to treatment effect on the understory vegetation.

4.3. Materials and Methods

4.3.1. Experimental Strategy

We excavated 20 intact soil monoliths (50 x 50 x 20 cm, L x W x D) from a beech forest understory and transported them to a greenhouse. After acclimatization to the greenhouse conditions, the monoliths were separated into soil moisture treatments (well-watered control and drought). To ease the logistics of the experiment, we performed two isotopic labeling events separated by 14 days. During the second labeling event, outside ambient temperatures increased, resulting in a rise in the average chamber temperature and a maximum chamber temperature of 50°C was recorded. We view this as a serendipitous event that provided us with an opportunity to test the effects of drought and drought plus strongly increased temperature (a heat-pulse) on the plant and soil microbial communities. Thus, our treatments (n=5) are well-watered control ©, drought (D), well-watered heat-pulse (H), and heat-pulse with drought (HD).

C and H treatment monoliths were watered constantly to field capacity, whereas the D and HD monoliths did not receive any water after the treatment onset. Due to the separation of the experiment into two stages, the acclimatization time of the soil monoliths to greenhouse conditions was 11 days before drought was initiated for the first labeling consisting of the C and D treatment, while the soil monoliths of the H and HD treatment had 25 days to acclimatize before the start of the drought treatment. The experimental treatments lasted a total of 28 days for all monoliths and the $^{13}CO_2$ labeling was performed on day 13 after the onset of the drought treatment.

4.3.2. Monolith Sampling and Set-up

The twenty soil monoliths were excavated with their natural understory vegetation in June from a managed beech (*Fagus sylvatica* L.) stand in the Hainich forest near Kammerforst, Germany (51°06'N, 10°23'E). The annual mean temperature and precipitation in our sample area are 6.5 – 7.5 °C and 750 – 800 mm, respectively. The soil types of the Hainich Forest are Luvisols and Stagnosols (Fischer *et al.* 2010) and the monoliths were sampled within a 100 m radius, thus assuring similar general soil properties (e.g., soil parent material, forest management influences). The monolith understory contained woodruff (*Galium odoratum*), young common ash (*Fraxinus excelsior*), and wood sorrel (*Oxalis acetosella*), among others (Table S 1).

In the field, the monoliths were placed in wooden boxes that were constructed with a drainage hole in the bottom. In the greenhouse, monoliths were placed underneath a shade cloth and quartz sand was used to fill in gaps along the edges between soil and wooden box. We measured the greenhouse air temperature continuously (T_{Air}; Kombisensor KS 550; ELV Elektronik AG, Leer, Germany), soil moisture content on all monoliths (ECH2O EC-5; Decagon Devices Inc., Pullman, WA,

USA), and soil temperature (T_{Soil}; Model 109 Temperature Probe; Campbell Scientific Inc., Logan, UT, USA) on a subset of monoliths (n=3). The soil moisture is given in mean-% values compared to the maximum water holding capacity (%max) for each treatment (n = 5). A light sensor (QSO-S PAR Photon Flux sensor; Decagon Devices Inc.) and a relative humidity sensor (VP-3 sensor; Decagon Devices Inc.) were installed under the shade cloth. We calculated soil pore water potential (kPa) by calibrating the soil moisture probe measure content against a pF curve (19.6 kPa). For values of pore water potential beyond the pF curve, we fit (Seki 2007) the pressure and soil moisture values to the model of Van Genuchten (1980). Soil $^{13}CO_2$ measurements were conducted by placing a CO_2 permeable membrane (8 cm, ACCUREL PP V8/2HF; Membrana GmbH, Wuppertal, Germany) vertically inside each monolith at 10 cm depth. The membrane was connected to a polyethylene tube placed vertically through the monolith. Soil gas pumped through the tubing (1 l min^{-1}) was monitored with a $^{13}CO_2$ cavity ring down spectrometer (Picarro G2101-I; Santa Clara, CA, USA).

4.3.3. Sample collection

Soil, aboveground plant tissues (pooled samples from leaves and stems), and coarse roots were sampled at 0, 6, 12, 14, 21, and 28 days after the drought began [Drought Day (DD) 0, 6, 12, 14, 21, and 28]. Soil samples were extracted from the Ah horizon using a cork borer (Ø 5mm). For $\delta^{13}C$ analysis of plant organic matter, we used plant material from all six sampling events; for ^{13}C-PLFA analysis, we used soil from five sampling events (without DD 6); and for 16S rRNA-based sequencing, we used soil from 3 samplings (DD 0, 14, 28). We sampled the plant species *Galium odoratum* for isotopic analysis because it was the most common plant growing on the monoliths. *Oxalis acetosella* was sampled when *Gallium odoratum* was absent. For all soil and plant samples, five randomly distributed subsamples from spatially different points within the monoliths were compiled. The samples were stored at -80°C until analysis.

4.3.4. Labeling

We performed 2 labeling events with 10 monoliths per event. After 2 weeks of drought, the vegetation of the monoliths was pulse-labeled with $^{13}CO_2$ on July 8 (1st event; labeling of C and D) and July 22 (2nd event; labeling of H and HD). A gastight chamber was placed over the monoliths to avoid leakage of $^{13}CO_2$ into the atmosphere. The $^{13}CO_2$ was produced by adding 80% H_3PO_4 (in excess) to 99% ^{13}C-enriched sodium bicarbonate (> 99.9% CO_2 with 99 atom-% ^{13}C; Cambridge Isotope Laboratories, Andover, MA, USA). In the second labeling event vegetation within the HD treatment wilted (pictures see Figure S 1), consequently, we increased the amount of label to ensure PLFA labeling (13 g versus 5 g used in the C and D treatment). Fans inside the roof dispersed the generated gas. The roof was removed after eight hours of $^{13}CO_2$ fumigation.

4.3.5. Isotopic analysis

Aboveground plant tissues, roots, and soil were dried for 48 h at 60°C, then ground to a homogenous powder. The isotopic composition of the bulk plant and soil samples was analyzed at the ZALF Isotope Core Facilities by combusting 0.3 – 0.5 mg of the ground material in an elemental analyzer (Flash HT Elemental Analyzer; Thermo Fisher Scientific, Waltham, MA, USA) coupled to an Isotopic Ratio Mass Spectrometer (Delta V Advantage IRMS; Thermo-Scientific). The isotopic values are expressed in delta notation (in ‰ units), relative to VPDB (Vienna Pee Dee Belemnite) and calibration was to IAEA-CH-6 (sucrose) and USGS40 (L-glutamic acid). Analysis of internal laboratory standards ensured that the estimates of the organic isotopic values were precise to within 0.1 ‰.

For phospholipid fatty acid (PLFA) extraction, soil samples were freeze-dried and 1 g of dry soil was extracted with a modified one-phase Bligh/Dyer method (Frostegård *et al.* 1991; Steger *et al.* 2011). The lipids were then separated into different lipid classes with increasing polarity (neutral, 28lycol- and phospholipids) using solid phase extraction with silicic acid columns (BondElut LRC-Si; Agilent Technologies Inc., Santa Clara, CA, USA). The fatty acid heneicosanoic acid (21:0) was added to the samples as an internal standard. The PLFA samples were dried and stored at -20°C until analysis. Quantification and identification of PLFAs were performed on a gas chromatograph (Steger *et al.* 2011). We used standard nomenclature to refer to the PLFAs (Boschker *et al.* 2005; Kaur *et al.* 2005; Denef *et al.* 2009; Steger *et al.* 2011).

The stable carbon isotopic composition of the individual PLFAs was determined on a Thermo-Scientific GC/C-IRMS system (Thermo Trace GC Ultra gas chromatograph coupled to a Delta V Advantage IRMS) at the UC Davis Stable Isotope Facility. PLFA $\delta^{13}C$ data were corrected for the addition of the methyl group by mass balance and were calibrated by our own internal and external fatty acid methyl ester (FAME) standards. Stable carbon isotope ratios are reported on the VPDB scale.

The ^{13}C uptake into the microbial PLFA biomass is expressed as excess $^{13}C_{PLFA}$ [µg C kg^{-1}]. The excess $^{13}C_{PLFA}$ represents the total amount of ^{13}C in the microbial PLFAs per kilogram soil and is calculated as follows (Fuchslueger *et al.* 2014):

$$\frac{(\text{atom\%}_{\text{Sample}} - \text{atom\%}_{\text{NA}}) * \text{Biomass [µgC]}}{100} * 1000\ [g] \qquad (1)$$

in which $\text{atom\%}_{\text{Sample}}$ is the atom% of the labeled PLFA sample, $\text{atom\%}_{\text{NA}}$ is the atom% of the PLFA sample one day before labeling (representing natural abundance of ^{13}C), and Biomass is the PLFA biomass [µg C].

4.3.6. RNA extraction and amplicon high-throughput sequencing

Total DNA and RNA were co-extracted from 100 mg soil (Ah horizon) (for extraction methods see Felsmann *et al.* 2015). We used MiSeq-based (Illumina Inc., San Diego, CA, USA) high-throughput sequencing to analyze the metabolically active (RNA-based) soil bacterial communities. We amplified cDNA samples with primers 8f and Eub518 targeting the V1 – V3 region of the bacterial 16S rRNA gene. At their 5' end, the reverse primers carried a specific 6-7 nt barcode and a 2 nt linker for each soil sample. The barcodes differed in at least 2 nt and were selected from those applied by Schloss *et al.* (2011). Two independent PCR reactions were performed using AccuPrime Taq High Fidelity (Invitrogen; Carlsbad, CA, USA). Cycling conditions were an initial denaturation of 1 min at 94°C, followed by 23 cycles of 20 s at 94°C, 30 s at 53°C and 90 s at 72°C, and a final extension of 7 min at 72°C. Combined amplicons were purified with the MSB Spin PCRapace kit (Invitek; Berlin, Germany), quantified using a Qubit fluorometer (Life Technologies; Darmstadt, Germany), and pooled to achieve a mixed sample with equimolar amounts of all PCR products. Adapter ligation and amplicon sequencing of 300 bp paired ends were carried out by GATC (Konstanz, Germany).

We used the software package Mothur v. 1.30.2 (Schloss *et al.* 2009) to process raw sequences. Paired sequences were used to make contigs and optimized by trimming off primer and barcode sequences (primer differences allowed, 2 bp, barcodes, 1 bp) and by removing sequences with mismatched nucleotides that differed by less than 6 units between the quality scores of both reads. To remove potential sequencing noise, reads differing by less than 1% of total residues were grouped by single linkage preclustering (Huse *et al.* 2010) and singletons were discarded as suggested in the UPARSE pipeline (Edgar 2013). High-quality reads were aligned using the SILVA database, and chimeras were removed using the Uchime algorithm (Edgar *et al.* 2011). After calculation of a distance matrix, operational taxonomic units (OTU) were generated using a cutoff of 0.03. For phylogenetic identification, the sequences were compared to the RDP 16S rRNA training set 10 using a confidence threshold of 80%. To equalize the number of sequences per sample, each group of sequences was subsampled to the size of the smallest group. Sequences were deposited in the NCBI Short Read Archive (SRP059718 and SRP059783).

4.3.7. Statistical analysis

Treatment differences in environmental (water holding capacity, soil water potential, soil temperature) and isotopic values (leaves, stems, roots) were tested using a repeated measures ANOVA followed by a Tukey's HSD test. Statistical analysis was carried out in R version 2.15.1 (R Development Core Team 2008). For the sequencing results, we used nonmetric multidimensional scaling (NMS) analysis to detect shifts in the bacterial community structure in which the relative proportion of OTUs within each sample was used as input for calculating NMS by PC-ORD v.6.08 (McCune & Mefford 1999). We implemented the Bray-Curtis distance measure to construct the NMS, which does not overemphasize the variance of low-abundant OTUs. Stress values were in the range of 8.2% and 9.2%, indicating a reliable test performance (Clarke 1993). We used a Multi-Response Permutation Procedure (MRPP) (Mielke & Berry 2007) to identify significant differences in the bacterial communities over time and between treatments. MRPP reports a chance-corrected within-group agreement (*A*), that describes the observed within-group homogeneity to the random expectation (i.e., $A = 1$ when communities within a treatment are identical and $A < 0$ when there is less agreement within the treatments than expected by chance (McCune and Grace, 2002)).

4.4. Results

4.4.1. Greenhouse conditions and soil water potential

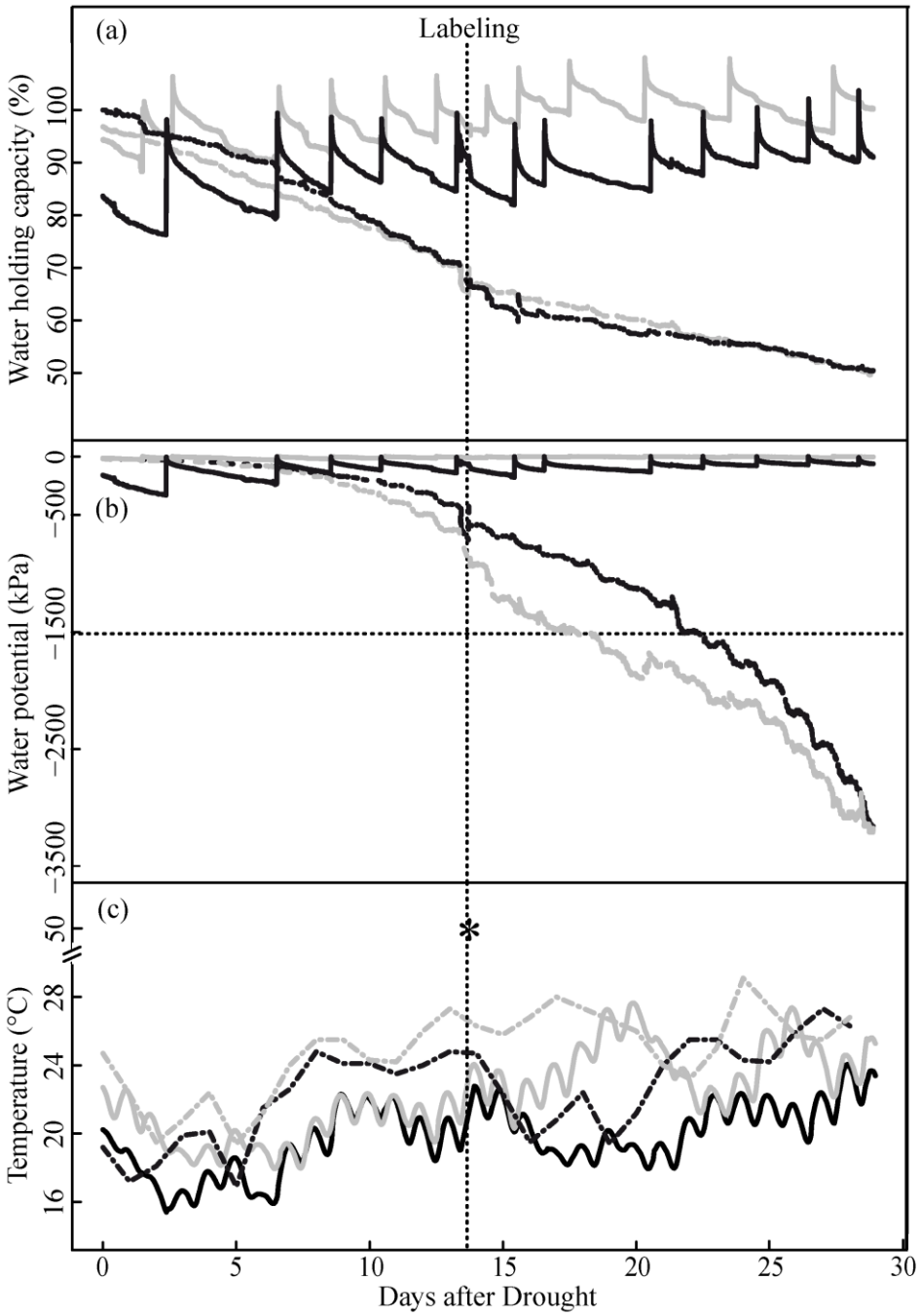

Figure 4-1: Water holding capacity (%), soil pore water potential (kPa) and temperature (°C) during the experiment: (a) percent of the mean maximum water holding capacity in well-watered control (C; black solid line) and heat (H; gray solid line) and non-watered drought (D; black dotted line) and heat-drought (HD; gray dotted line); (b) soil pore water potential (kPa) during the experiment (legend as above) with horizontal dotted line indicating permanent wilting point and (c) air temperature in dotted lines [Tair; daily mean value of 15 min interval measurements (°C)] and soil temperature in solid lines [hourly Tsoil (°C)] for C and D (black) and H and HD (gray); n = 5 for each treatment. * Indicates the maximum temperature inside the roof during the second labeling.

Figure 4-1a and b show the soil moisture (SM) as water holding capacity (%) and water potential (Ψ) in kPa over the experiment. At the beginning of the experiment, all treatments were at or near 100% $_{max}$ (SM) and soil water potential was near 0 kPa, indicating that all treatments were well watered. The C and H treatment remained at these levels throughout the duration of the experiment. In the D and HD treatments, the soil moisture and water potential gradually decreased from the beginning of the experiment and SM reached 65%$_{max}$ at the time of labeling (after 2 weeks of drought). Permanent wilting point in D was reached after DD 22 and in the HD treatment after DD 18. By the end of the 28-day experiment, SM was reduced below 48% $_{max}$ and Ψ below -3250 kPa in both treatments.

Mean daily air temperatures (T_{air}) in total varied between 17 and 31°C during the experiment and mean daily soil temperatures (T_{soil}) between 16 and 27°C (Figure 4-1c). During the H and HD treatment, an increased air and soil temperature were observed over time. Nine days (from 28) were significantly warmer by at least 4.6°C ($P < 0.001$) when compared to the C and D treatment and 23 days had significantly warmer soil temperatures by 1 to up to 7°C ($P < 0.05$ and < 0.001). During the second labeling, the temperature inside the chamber substantially increased (up to 50°C) as the labeling was done outside the greenhouse with ambient temperatures approaching 40°C by midday. Therefore, we increased the available concentration of $^{13}CO_2$ to compensate for the reduction in the functional leaf area (Figure S 1) that occurred during the heat-pulse; however, given the relative similar values in label of the D and HD plant samples, we infer that plant uptake, and not the amount of $^{13}CO_2$, is what ultimately controlled the amount of label in the plant-soil continuum.

4.4.2. Plant and soil isotopic patterns

Before labeling, mean $\delta^{13}C$-values ranged from -32.6‰ to -31.1‰ in leaf and stem tissues and from -32.5‰ to -30.3‰ in roots. Compared to the natural abundance levels before labeling, all treatments were significantly enriched in ^{13}C ($P < 0.05$) (Figure 4-2a). Comparisons across the treatments show that C and H followed the same label dynamics, while there was a significant difference between C and D initially (DD 14), and a significant difference between HD and D on DD 21 ($P < 0.05$). The $\delta^{13}C$ in aboveground tissue tended to decrease between the day after labeling (DD 14) and 7 days later (DD 21); values decreased by 61.2% (C), 62.2% (D), 40.4% (H), and 8% (HD), but only the decrease in the control was significant ($P < 0.01$). The $^{13}CO_2$ canopy fumigation successfully labeled root biomass within all treatments (pre vs. post label, $P < 0.01$). However, the increase was not significant between the treatments (Figure 4-2b), most likely due to the high spatial variability of the label within the monoliths. The maximum $\delta^{13}C$-value (146.33‰ ± SE 25‰) for the roots was found in the control 15 days after labeling (DD 28).

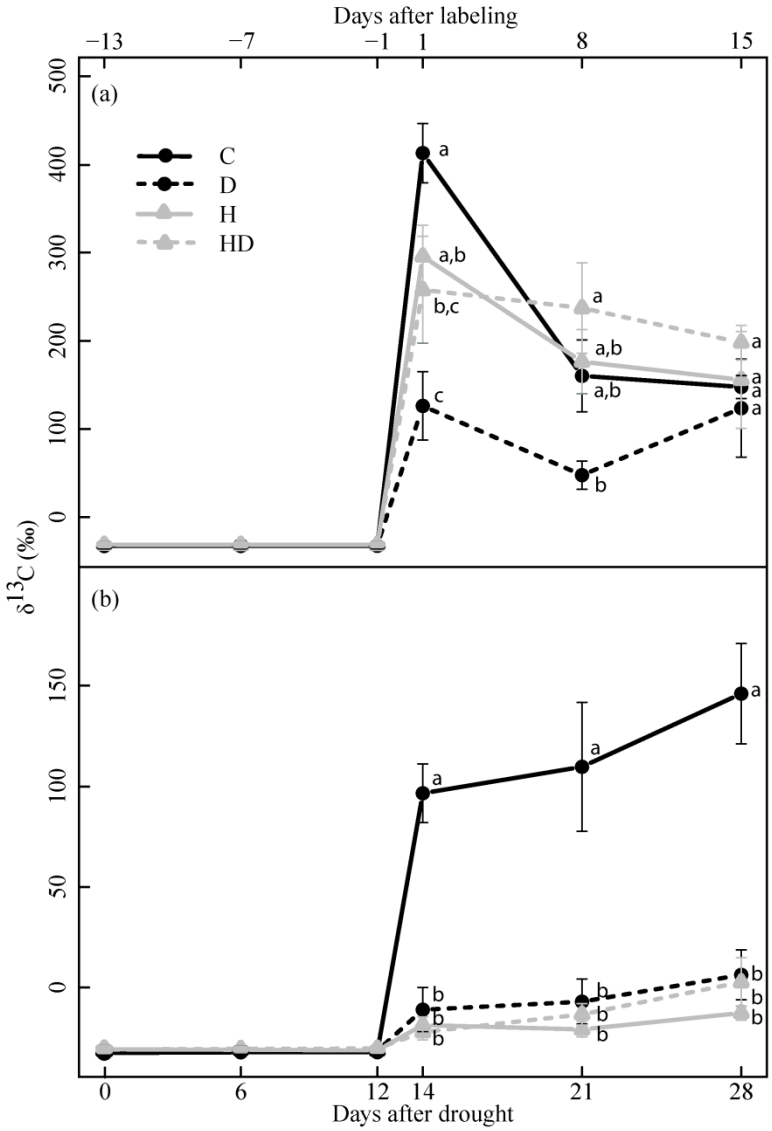

Figure 4-2: Fate of the ^{13}C label for (a) aboveground tissues (leaves and stems) and (b) roots of *Galium odoratum* in the control (C), drought (D), heat-pulse (H), and heat-pulse with drought (HD) treatments as $\delta^{13}C$ values (‰) during the experiment – with the upper x-axis showing days before (negative values) and days after labeling (positive values) and the lower x-axis showing days after the onset of the drought treatment. Values are means ± SE (n = 5). Significances are indicated separately for each time point.

We monitored $^{13}CO_2$ isotopic composition in the labeling chamber during the labeling and in the soil gas for 5 days after labeling. The purpose of these values was primarily to assess the arrival of label rather than to quantify fluxes. In general, we could detect a pulse of labeled carbon present in soil $^{13}CO_2$ after 1 day in C and after 2 days in HD (Figure S 2). We could not determine the soil $^{13}CO_2$ dynamics during the 8-h labeling or directly after labeling so we cannot account for changes during this time. However, changes in isotopic composition of soil organic carbon in response to the ^{13}C labeling could not be observed over time or between the treatments (data not shown).

4.4.3. Effects of drought and/or heat on soil microbial groups and linkage to the plant-soil carbon continuum

In general, label was incorporated into microbial and fungal PLFAs indicated by ^{13}C excess values greater than 0 (Figure 4-3). For microbial PLFAs, the HD treatment had the largest negative impact indicated by the least ^{13}C excess values. Fungal PLFAs (c18:1ω9c) performed well in H, incorporating more label when compared to the C and D treatment.

We analyzed 16 PLFAs to evaluate different groups of microorganisms (see Table S 2 for a list of used PLFAs). The remaining PLFAs that had been extracted did not yield sufficient material for analysis (i.e., they were below the detection limit).

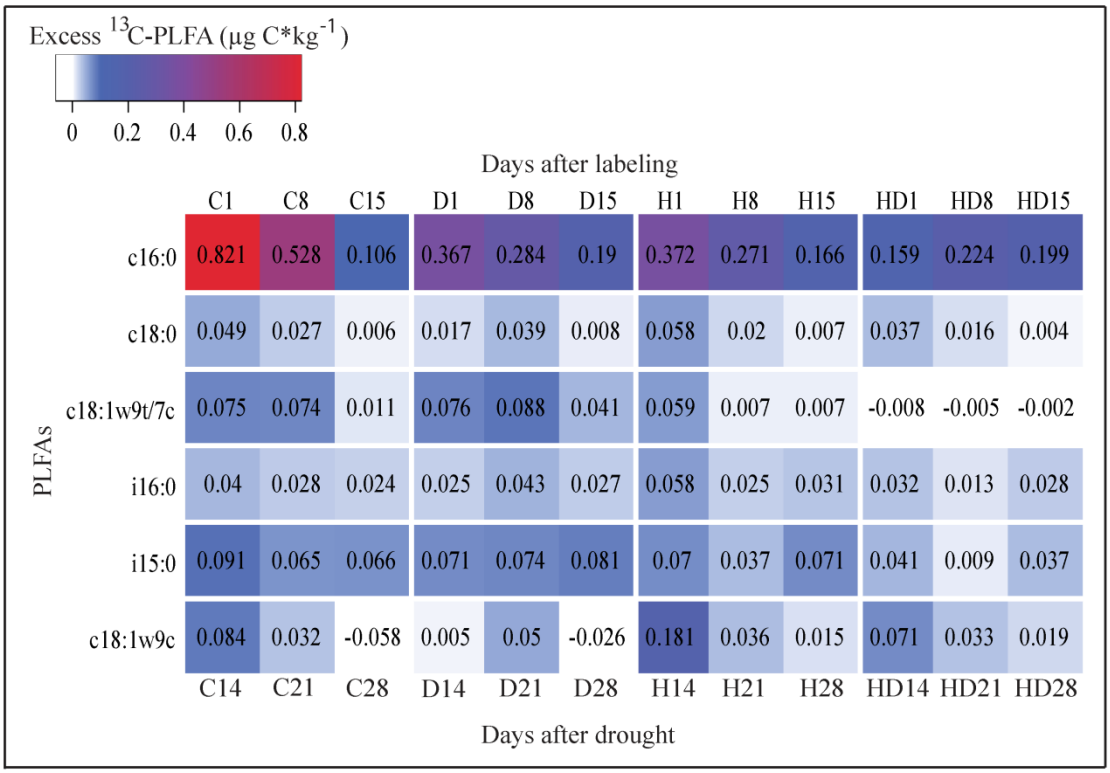

Figure 4-3: Transfer of ^{13}C label to PLFAs. The heatmap shows the excess ^{13}C-PLFA values (µg C×kg^{-1}) during the experiment for control (C) and treatments (D, H, HD) for different PLFAs. The upper x-axis displays days after labeling and the lower x-axis days after the onset of the drought treatment. Values are means (n = 5).

We chose PLFAs from each marker group except *Actinomycetes* and expressed the calculated excess ^{13}C-PLFA values in a heatmap (Figure 4-3). High ^{13}C excess values for general PLFA markers were found in c16:0, c18:0, and c18:1ω9t/7c with c16:0 having the highest values with a maximum of 0.821 ± SE 0.189 µg C x kg^{-1} 1 day after labeling (DD 14) in the control. There was a significant difference between 1 and 15 days after labeling (DD 14 and 28) for the general bacterial biomarker c16:0 ($P < 0.01$) when considering all the treatments (Figure 4-3). H and HD (but not D) were significantly different to C ($P < 0.05$ and 0.001, respectively).

There were no significant differences in time and/or treatment for the PLFA marker of gram-positive bacteria (i16:0), but the excess values were positive, indicating that ^{13}C was incorporated. The PLFA marker i15:0 (which represents heterotrophic bacteria) had the same significant differences between treatments as c18:1ω9t/7c with HD being significantly different to C and D ($P < 0.05$). The fungal PLFA c18:1ω9c showed higher values in H which was significantly different from C as well as D ($P < 0.05$) and this marker showed a significant decrease over time when considering all treatments.

4.4.4. Effects of drought and/or heat on the bacterial community structure

We used the MiSeq-based sequencing of the V1 – V3 region of the 16S rRNA gene to analyze the metabolically active (RNA-based) bacterial communities from five replicates per treatment. In total, 8,209,608 high-quality full-length reads were obtained. All groups of sequences were subsampled to 42,368 reads each, which was the size of the smallest sample. The total number of OTUs was 23,709 per sample, the number of OTUs ranged from 2748 to 4642. Based on the 42,368 16S rRNA sequences per sample, a reasonable coverage of 96.6%, the mean for all samples, was achieved.

The inverse Simpson (1/D) diversity index was used to evaluate changes in bacterial diversity (Table 4-1) within treatments. The diversity did not change significantly within C, and we also observed only negligible changes in H and D. Similarly, species richness (number of OTUs), remained unchanged within control and treatments (Table 4-1). However, diversity in HD significantly decreased over time ($P < 0.05$).

Table 4-1: Richness and diversity (mean ± SE) of OTUs based on 16S rRNA sequences for treatments at DD 0, 14 and 28 (n = 5). The diversity index is the inverse Simpson (1/D). ^{a,b,c} indicate significant differences between treatments. * indicates significant differences between sample collection.

Treatment		Control ©			Drought (D)	
Drought Day	0	14	28	0	14	28
OTU Richness	4038±142^{a}	4097±167^{a}	4065±166^{a}	3702±198^{a,b}	3759±149^{a,b}	3921±254^{a,b}
InvSimpson	165.3±13.2^{a}	140.4±8.8^{a}	145.5±4.7^{a}	130.3±7.2^{a,b}	109.7±5.7^{a}	137.0±17.0^{a,c}
Treatment		**Heat (H)**			**Heat-Drought (HD)**	
Drought Day	0	14	28	0	14	28
OTU Richness	3635±57^{c}	3328±170^{c}	3243±130^{c}	3479±95^{b,c}	3432±142^{b,c}	3756±157^{b,c}
InvSimpson	103.3±4.2^{b}	131.3±18.0^{a}	92.2±4.7^{b,c}	104.8±7.4^{b}	56.6±6.4^{b,*}	56.1±4.3^{b,*}

An NMS-based ordination for the identified OTUs was used to visualize the variability in the bacterial community and the differences in community structure between the treatments (Figure 4-4). D and C, as well as H and HD had a similar community structure at DD 0. The community composition changed over time in C, most likely due to sampling during the tail end of the acclimation period exemplified by the relative low *A*-value (Table 4-2). The bacterial community composition in H remained unchanged, but we found a distinct shift between DD 0 vs. DD 14 and DD 28 for D and HD, with a strong initial community shift for the latter treatment. MRPP analysis further supports a clustering that distinguishes between the well-watered and drought treatments (Table 4-2). Thus, we found a similar trend of the community shifting between D and HD even though HD obviously had a stronger effect on the community. Remarkably, apart from the two shifts in the D and HD treatment, the bacterial community structure displayed a high tolerance.

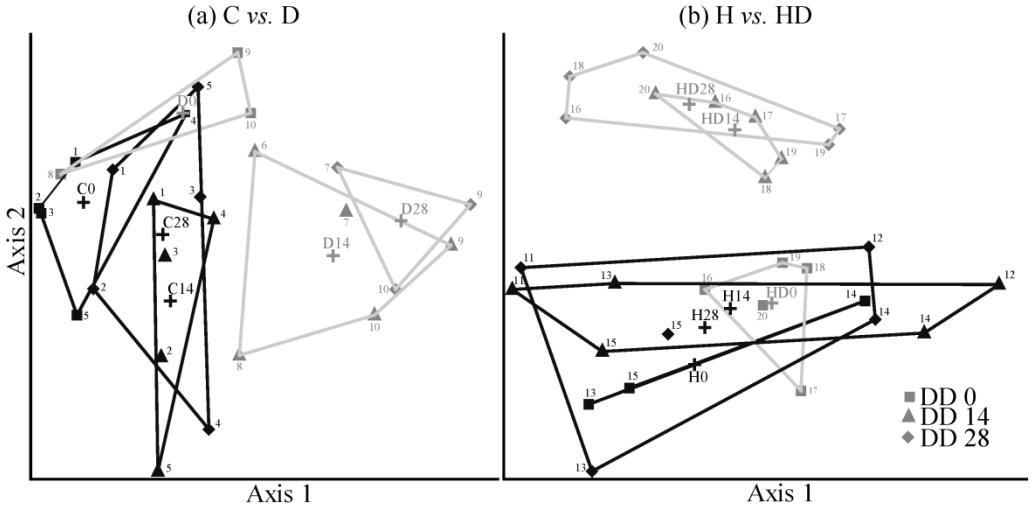

Figure 4-4: Effects of drought, a heat-pulse and heat-pulse with drought on the soil bacterial community structure. NMS ordination plots of the bacterial community structure at three different time points (DD 0, 14 and 28) for (a) control (C, black lines) vs drought (D, gray lines) and (b) heat (H, black lines) vs. heat-drought (HD, gray lines). Numbers at points indicate different monoliths from which samples were taken; centroids are indicated by +.

Variability within the C and H treatment was largely due to the different monoliths. The community structure of a single monolith often clustered closely together over time; this was especially prominent for the H treatment. Thus, the community structure of monoliths 11 – 15 was comparable over the whole period. Even in the HD treatment where a strong shift occurred between DD 0 and DD 14, the community structure was again similar for the single monoliths 16 – 20 at DD 14 and DD 28 (Figure 4-4).

Table 4-2: Significance test (MRPP) of the effect of drought, a heat-pulse and a heat-pulse with drought on the bacterial community structure. *A*- and P-values for control © and treatments (D, H, HD) from the comparison of different time points (DD 0, 14 and 28) are given. Bold numbers indicate a significant *A*-value.

	A	**P-value**
C0:C14	**0.13**	0.021
C0:C28	0.05	0.181
C14:C28	-0.04	0.667
D0:D14	**0.26**	0.007
D0:D28	**0.32**	0.029
D14:D28	0.01	0.391
H0:H14	-0.06	0.671
H0:H28	-0.06	0.626
H14:H28	-0.08	0.988
HD0:HD14	**0.34**	0.002
HD0:HD28	**0.32**	0.004
HD14:HD28	0.06	0.148

The analysis of phylotypes showed a high phylogenetic diversity with a total of 22 phyla. Dominant phyla were *Proteobacteria* (46%), *Actinobacteria* (18.4%), *Planctomycetes* (12.4%), and *Acidobacteria* (10.8%). We observed an increase in *Proteobacteria* in the D and HD treatment, while *Planctomycetes* decreased. *Actinobacteria* decreased only in HD. The phylotypes were analyzed to reveal taxonomic groups, which intensively responded to the treatments. In total, 31 phylotypes with a relative abundance of more than 0.1% of the bacterial community could be detected that were increased or decreased by more than 50% over time (Figure 4-5). The phylotypes could be grouped based on their response to the treatments (decrease and increase in relative abundance which can be seen in the hierarchical clustering) in correspondence to their taxonomic assignment. As already seen in Figure 4-5, we could detect a clear trend between bacterial phyla. Phylotypes belonging to the α-, β-, and γ-*Proteobacteria*, *Verrucomicrobia*, and *Firmicutes* increased in relative abundance in the D and HD treatment. *Actino*- and *Acidobacteria* groups decreased only under HD and *Planctomycetes* decreased in both, D and HD. The response of these phylotypes to D and HD were consistent; thus, this response-characteristic seems to be phylogenetically highly conserved.

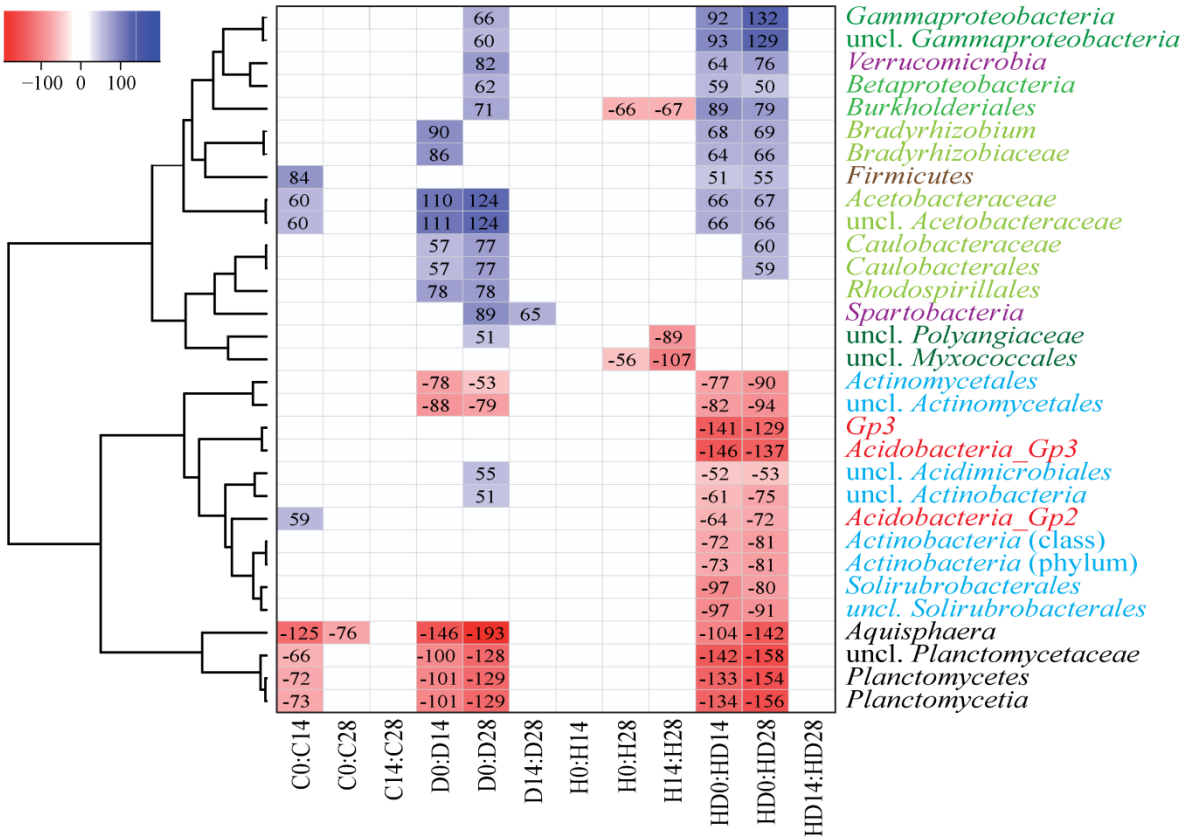

Figure 4-5: Phylotypes with abundance shifts in control © and treatments (H, D, HD) between three different time points (DD 0, 14, 28). Shown groups had a relative abundance of more than 0.1% of the bacterial community and increased or decreased by more than 50% over time. Values are given in % increase (blue) or decrease (red) as means. Light green to dark green = *Alpha-* to *Deltaproteobacteria*, violet = *Verrucomicrobia*, brown = *Firmicutes*, blue = *Actinobacteria*, red = *Acidobacteria*, black = *Planctomycetes.*

4.5. Discussion

We designed our experiment to capture the plant-soil carbon continuum and soil microbial community responses to drought and temperature extremes. We imposed a month-long drought in which no water was supplied, and a heat-pulse (at DD 13) that lasted 8 hours with maximum air temperatures of approximately 50°C. The soil moisture stress we induced exceeded even the strong drought conditions for this part of Germany (Gimbel *et al.* 2015). Soil water potential decreased far below the permanent wilting point, a plant physiological threshold, and below the point at which carbon substrate diffusion halts in soils, a microbial environmental limitation (Manzoni *et al.* 2012). The heat-pulse temperature was short though extreme, inducing wilting and foliar damage (Figure S 1) and may approach the temperature range of recent heat waves in Europe (Berard *et al.* 2011).

Importantly, both the plant-soil carbon continuum and the microbial communities from soil monoliths exposed to drought (D) and a heat-pulse with drought (HD) changed significantly, thus meeting the requirement of Smith (2011) that an extreme climate event should result in a shift in ecosystem biological characteristic. Overall we found that our treatments did not completely sever the linkages between plants and soil; however, contrasting $\delta^{13}C$ patterns between above- and belowground tissues, as well as the ^{13}C in PLFAs, suggest strong alterations in the linkage between photosynthesis and belowground processes among the treatments. The microbial community dynamics analyzed by PLFAs and high-throughput sequencing of the 16S rRNA were most strongly affected by the combination of a heat-pulse and drought (HD).

4.5.1. Plant-soil carbon continuum

We ^{13}C labeled new plant assimilates and tracked the label through the plant-soil microorganism continuum as a proxy for the strength in coupling between aboveground plant tissues and belowground communities. As the source of labeled carbon, leaf uptake reflects the initial impact of the treatments on plants and their potential to deliver recent assimilates belowground. All treatments showed a strong ^{13}C increase in leaves 1 day after labeling (DD 14), indicating that photosynthesis was still functional despite the water and heat stress. This stress was exemplified by the significant differences between the C and D treatments. Plants experiencing drought can place a priority on maintaining hydraulic functioning (Hartmann *et al.* 2013; Sevanto *et al.* 2014), for example, by closing their stomata, which then leads to reduced carbon assimilation. A consequence of drought can also be decreased phloem loading and lower phloem transport velocity (Ruehr *et al.* 2009).

The corresponding belowground patterns were different than those aboveground. The pulse of labeled carbon was present in soil CO_2 after 1 day in C and after two days in HD (Figure S 2). Based on the pattern in the HD treatment, we can infer that plants were under water stress resulting in a delay of delivery of new assimilates used for root and rhizosphere respiration (Ruehr *et al.* 2009; Burri *et al.*

2014). However, in all treatments, root tissue tended to increase in ^{13}C over time, indicating that labeled carbon had been allocated belowground.

The carbon continuum remained intact, although weakened, and microbial communities took up labeled carbon as well, but differences in PLFA label amount between treatments attest to their effects on the linkage between plants and soil microbial communities, thus confirming hypothesis 2. Based on the response in the control monoliths alone, there is clear indication that the potential for carbon delivery to soil microbes is high, despite sampling at a spatial scale larger than the rhizosphere. When we consider the excess patterns of the general PLFA marker c16:0, which was the most enriched across all treatments, we can see that within the drought treatments the HD monoliths were more severely negatively affected.

Thus, to address Hypothesis 1, based on the arrival of label in all plant tissues and representative PLFAs, we can assert that none of the treatments were too severe to totally disrupt within-plant transport over the experiment (despite wilting in D and HD), and the depletion of the aboveground signal along with the concurrent enrichment of the belowground root tissue suggests that carbon sinks belowground were still active (Koerner 2011; Hasibeder *et al.* 2015). However, the stress treatments severely impaired the carbon continuum, which is readily apparent when comparing the root label patterns of the control to the stress treatments. Ultimately, the amount of label may not signify the ecological relevance of the severely impacted carbon continuum we observed; the relevance, rather, lies in the degree to which the microbial community was affected.

By linking the root and the PLFA label dynamics, we can detect a possible change in assimilate allocation patterns. The PLFA ^{13}C excess was generally higher in the control than in the treatments based on absolute values [e.g., c16:0 1 day after labeling (DD 14)], but the picture changes when the PLFA label is compared to the ^{13}C enrichment in roots (Table 4-3). The ^{13}C label in roots was 6.2 times higher in C compared to D 1 day after labeling, in comparison, the c16:0 PLFA of C exceeded D by only a factor of approx. 2.2. Similar relations were found for the H and HD treatments (Table 4-3). This clearly indicates that a relatively higher proportion of new assimilates arriving in roots was transferred to soil microorganisms in the drought- and heat-stressed treatments.

Table 4-3: Relative level of ^{13}C incorporation between carbon pools (roots and the general PLFA marker c16:0) 1 day after labeling between experimental treatments. A more equitable ratio between the compared treatments indicates the "potential" of available carbon reaching belowground pools; in all cases the PLFAs received the largest portion of labeled carbon.

C-Pool	C:D	C:H	C:HD
Roots	6.2	9.8	14.8
c16:0 PLFA	2.2	2.2	5.1

Based on these PLFA and root patterns, we infer two pathways of carbon flow to belowground microbial communities. The first pathway is evidenced from the strong increase of label in the plant organs and ^{13}C excess of PLFAs directly after labeling during non-stressed conditions, while the second pathway is apparent during environmental stress, in which the microbial pool received a higher relative proportion of labeled carbon. This may be indicative of different sink strengths or sink priorities of the carbon pool that different microbial communities (e.g., mycorrhizae) are associated with. A similar finding was observed by Hasibeder *et al.* (2015), who found that under control conditions, recent assimilates were directed to root respiration, while root respiration from plants under stress was fueled by stored carbon, possibly allocating recent assimilates to alternative belowground sinks. While assimilates may be used for root respiration under well-watered conditions or osmotic adjustment during the onset of water-stressed conditions, our results suggest that the microbial belowground community may exert a stronger sink strength during drought in which soil moisture levels exceed the plant wilting point.

4.5.2. Microbial community structure

Overall, we found the dominant taxonomic groups in our treatments were similar to other studies on forest soil (Dimitriu & Grayston 2010; Sun *et al.* 2014; Felsmann *et al.* 2015), consisting of *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, and *Acidobacteria*. However, based on our high-throughput sequencing of the 16S rRNA, we observed multiple trajectories in the community shifts due to the stress treatments. *Planctomycetes* had the strongest negative response in our experiment (strong changes in abundance resulting in decreases under D and HD). The phylum *Proteobacteria* performed best under the treatments with increases in relative abundance in D and HD, possibly due to a generally high soil organic carbon availability (Fierer *et al.* 2007; Sun *et al.* 2014) in our forest soil. The H treatment alone had no detectable effect, D had a relatively small effect, but the HD treatment had the strongest effect on the bacterial community structure with a decrease in bacterial diversity. Furthermore, most of the changes in the bacterial community occurred within the first two weeks of treatment, indicating that an environmental threshold might have been reached for the bacterial community.

Our findings suggest that microbial communities can tolerate a heat-pulse alone. We did not observe a significant shift in the OTU richness, diversity, and community structure or changes in relative abundance of the phylotypes in H. Castro *et al.* (2010) found that warming does not always lead to predictable or significant changes in bacterial and fungal abundance or community structure, while Schindlbacher *et al.* (2011) suggest that heat affects major groups of soil microbial communities only when other limitations are present (e.g., water or nutrient limitation). Over a long time period of selection and evolution, microorganisms have adapted to tolerate and survive stress

through a variety of different strategies (Schimel *et al.* 2007; Wallenstein & Hall 2012; Barnard *et al.* 2013; Griffiths & Philippot 2013). Thus, our heat-pulse was not strong enough to see an immediate effect. This is congruent to the high tolerance that we found for the microbial community in our soils; however, even though we did not observe a change in the bacterial community structure in the H treatment, a delayed stress response is still possible (e.g., through large shifts in the allocation of carbon and nitrogen) (Schimel *et al.* 2007).

The bacterial communities were more or less tolerant to the D treatment, contradicting our original expectation, and we found only minor changes in the community structure relative to the control. Furthermore, we were not able to detect significant differences in richness and diversity of the bacterial communities within this treatment. Over the course of the experiment, we found that the relative abundance of the phylum *Proteobacteria* increased and *Planctomycetes* decreased, but we observed a similar though lower trend in C. We infer from these patterns that species that performed poorly under drought (e.g., bacteria from the phylum of *Planctomycetes*) were out-competed by more tolerant species (e.g., from the phylum of *Proteobacteria*), and thus a diversity or species richness change was not observed within the shifting community. There was also a slight yet significant increase in the *Actinobacteria* relative abundance in D. Numerous members of *Actinobacteria* are known to compete well under dry conditions (Barnard *et al.* 2013; Felsmann *et al.* 2015). Filamentous (mycelium-forming) *Actinobacteria* use this growth form to facilitate growth and expansion under conditions of low hydraulic connectivity (drought conditions) in unsaturated soils (Wolf *et al.* 2013), which could be an explanation for stimulated growth under moderate drought.

The mixed response of bacterial communities to drought in our experiment clearly reflects the different physiological strategies (often accompanied by a change in community composition) microorganisms have developed to cope with drought stress. Physiological strategies for drought include production of protective molecules, dormancy, or higher carbon use efficiency (Schimel *et al.* 2007). Soil microorganisms also have on average a relatively dry optimum (-320 kPa) and are capable of respiring even under lower water potentials (-2000 MPa) displaying a broad range of moisture tolerance (Lennon *et al.* 2012). However, our study imposed extreme environmental conditions carried out over several weeks and thus only gives a short-term perspective of microbial community shifts. Over the long-term, microbial community shifts may also be driven by ecosystem feedbacks to drought, such as changes in soil C/N ratio, pH, and nitrogen input (Evans *et al.* 2013).

The strongest change in the active microbial community was in the HD treatment, which had a clear negative effect on diversity (only in HD did we see a significant decrease in diversity) and resulted in a distinct shift in the community structure and changes in relative abundance of many phylotypes. In the HD treatment, *Planctomycetes*, *Actinobacteria*, and *Acidobacteria* were the phylotypes most affected and had the largest decrease in relative abundances. Interestingly, many of

the starkest changes in abundances occurred in HD in which the plant community suffered the most; furthermore, these changes did not occur in C, D, or H. We infer from this finding that the indirect effects of the treatment on the microbial pattern resulted in the largest community shift, confirming hypothesis 3; however, our experimental design excludes the possibility to test solely for direct effects of the treatment on the microbial communities. This pattern also corresponds clearly to the low level of label in the ^{13}C PLFAs we found in HD compared to the other treatments. Thus, our data suggest that *Actinobacteria* and members of *Acidobacteria* are more tightly linked to the fate of plants and their carbon delivery during environmental stress (i.e., the indirect climate change effect). Furthermore, the corresponding increase in relative abundance of *Alpha*- and *Gammaproteobacteria* suggests that members within these phyla were able to take advantage of the altered belowground conditions that occurred with the plant stress response (e.g., reduced carbon transfer belowground).

Our results reinforce current observations of a diverse microbial response to environmental stress in which members of various phyla exhibit optima during moderate to dry moisture conditions (Lennon *et al.* 2012; Barnard *et al.* 2013). Given that a subset of phyla responded similarly (i.e., in trajectory but not magnitude) in D and HD, we can conclude that the resistance mechanisms under the direct climate change effects of drought and increased temperature are phylogenetically highly conserved. In the same breath, our understanding of the microbial response to environmental stress has also expanded. Our study exemplifies that many of the shifts in the microbial communities that we might expect from climate change will result from the plant-soil microbial dynamics rather than from direct effects on soil microbes alone. In particular, the plant's role in carbon delivery belowground is critical for some phyla, and as our data suggest, these microbes may maintain belowground pools as a carbon-sink priority even for stressed plants. The plant-soil microorganism relationship is fundamental to terrestrial ecosystems globally, and advances in understanding or predicting balances of energy and nutrient fluxes or alterations of microbial diversity under global climate change will clearly depend on our ability to accurately characterize this relationship.

4.6. Acknowledgments

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5. Plant-microbe interactions under drought in an aquatic ecosystem

Desiccation of sediments affects assimilate transport within aquatic plants and carbon transfer to microorganisms

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5.1. Abstract

With the projected increase in drought duration and intensity in future, small water bodies, and especially the terrestrial-aquatic interfaces, will be subjected to longer dry periods with desiccation of the sediment. Drought effects on the plant-sediment microorganism carbon continuum may disrupt the tight linkage between plants and microbes which governs sediment carbon and nutrient cycling, thus having a potential negative impact on carbon sequestration of small freshwater ecosystems. However, research on drought effects on the plant-sediment carbon transfer in aquatic ecosystems is scarce. We, therefore, exposed two emergent aquatic macrophytes, *Phragmites australis* and *Typha latifolia*, to a month-long summer drought in a mesocosm experiment. We followed the fate of carbon from leaves to sediment microbial communities with $^{13}CO_2$ pulse labeling and microbial phospholipid-derived fatty acid (PLFA) analysis. We found that drought reduced the total amount of carbon allocated to stem tissues but did not delay the transport. We also observed an increase in accumulation of ^{13}C-labeled sugars in roots and found a reduced incorporation of ^{13}C into the PLFAs of sediment microorganisms. Drought induced a switch in plant carbon allocation priorities, where stems received less new assimilates, leading to reduced starch reserves whilst roots were prioritized with new assimilates, suggesting their use for osmoregulation. There were indications that the reduced carbon transfer from roots to microorganisms was due to the reduction of microbial activity via direct drought effects rather than to a decrease in root exudation or exudate availability.

5.2. Introduction

Due to climate change, drought events are expected to increase in duration and intensity in the near future (Beniston *et al.* 2007; Briffa *et al.* 2009; IPCC 2012; Reichstein *et al.* 2013; Bahn *et al.* 2014). There have been many studies that assess drought stress responses of terrestrial plants, with a focus on carbon transport and plant-soil interactions (Knorr *et al.* 2008; Barthel *et al.* 2011; Reyer *et al.* 2013; von Rein *et al.* 2016), but only a few have covered emergent aquatic macrophytes (Li *et al.* 2004; Pagter *et al.* 2005), despite their relevance for aquatic-terrestrial ecosystem functioning (Downing *et al.* 2006; Werner *et al.* 2013).

Emergent aquatic macrophytes face many environmental challenges such as continuous flooding and/or periods of falling dry and increasing drought stress due to climate change (Chaves *et al.* 2002). When the sediment is submerged, macrophytes need to transport oxygen to roots and rhizosphere internally from aerial organs using large air spaces like aerenchyma channels and pith cavities (Brix 1994; Engloner 2009).They also have adapted to stress from varying water regimes by, among other life strategies, adjusting stomatal functioning and RuBisCO activity (Li *et al.* 2004). Macrophytes are of general importance for freshwater ecosystems as they provide a huge surface area for microbial communities as well as habitat for wildlife (and support its diversity), stabilize sediment surfaces and transfer oxygen to the rhizosphere, which creates small areas with oxidized conditions where aerobic organic matter decomposition is possible (Brix 1994; Perrow *et al.* 1999). However, while well adapted to flooding, emergent aquatic macrophytes are often assumed to be susceptible to drought, which reduces photosynthesis, biomass production, and stomatal conductance (Li *et al.* 2004). Drought stress not only reduces carbon assimilation but can also lead to changes in the carbon partitioning within plants, resulting in reduced and delayed transport of recent assimilates to roots (Ruehr *et al.* 2009; von Rein *et al.* 2016). Reduced assimilate export from and increased sugar accumulation in leaves have been linked to the adjustment of osmotic potential, leading to changes in non-structural carbon (NSC) concentrations in plants (Sánchez *et al.* 1998; Pagter *et al.* 2005; Peuke *et al.* 2006). As an alternative explanation to osmotic adjustment, increased leaf, but also root, sugar concentrations at the beginning of a drought period have been argued to be an indication of reduced sink tissue activity before photosynthesis acclimates (McDowell *et al.* 2011).

Such drought effects on plant functioning are especially relevant for small freshwater ecosystems like ponds and kettle holes (small, shallow standing freshwater systems; Reverey *et al.* 2016), with a high perimeter-to-area ratio that emphasizes the importance of the littoral where (i) emergent macrophytes play a central role for the system's carbon balance, and (ii) prolonged dry periods can lead to shifts from permanent to temporary water bodies and thus extensive sediment desiccation (Werner *et al.* 2013; Reverey *et al.* 2016).

A reduced belowground carbon transport upon drought also negatively affects the carbon continuum between plants, their rhizosphere, and free subsurface microbial communities (von Rein *et al.* 2016). The link between aquatic macrophytes and rhizosphere microorganisms is particularly close because the latter not only depend on the rhizodeposited plant carbon but also on the redox environment created by the plants, with aerobic microbial groups depending on oxygen leakage from roots (Brix 1994; Holguin *et al.* 2001). Therefore, these plants can be seen as a direct link between the atmosphere and aquatic sediments.

If the coupling of the assimilate flux within the plant and from the plant to the rhizosphere in aquatic sediments is severed, microorganisms will face a reduction of the carbon and energy supply in addition to the direct osmotic effects caused by water restriction. Thus, if the tight link between aquatic macrophytes and microorganisms is disrupted, it is possible that microbial community composition and functioning will be affected, as previously observed in terrestrial systems (e.g., Fuchslueger *et al.* 2014; von Rein *et al.* 2016). However, studies on interactions between wetland plants and microbes are scarce and the mechanistic link between above- and belowground community structure is not well understood so far (Gutknecht *et al.* 2006). It is vital to understand plant-microorganism interactions under future climate scenarios as microorganisms, in turn, affect plant nutrient availability and thus productivity (Schnitzer *et al.* 2010; Schnitzer & Klironomos 2011; Wagg *et al.* 2014), and also significantly trigger greenhouse gas emissions through organic matter decomposition in the sediment (Brix *et al.* 2001).

We assessed above-to-belowground interactions by investigating the drought response and the plant-sediment microorganism carbon coupling of two cosmopolitan wetland species, *Phragmites australis* and *Typha latifolia*. Using a $^{13}CO_2$ pulse labeling approach under controlled conditions in mesocosms, we aimed to characterize the partitioning of recent ^{13}C-labeled assimilates to different plant compartments (leaves, stems, and roots) as well as the transport to rhizo-dependent microorganisms by assessing the incorporation of ^{13}C into phospholipid-derived fatty acids (PLFAs) of the microorganisms in both plant species. We hypothesized (1) that drought impairs the carbon transport within plants, causing a reduction in the amount of carbon allocated and a reduction in carbon transport velocities belowground. In addition, we assumed that carbon partitioning is driven by osmotic adjustment as well as by the reduction of the belowground sink activity due to a reduced demand of roots through impaired growth and metabolic activity. Furthermore, we hypothesized (2) that the drought reduces the ^{13}C label incorporation into microbial PLFAs, with drought effects on microorganism carbon metabolism potentially being both direct and indirect through reduced carbon delivery from the plant.

5.3. Material and Methods

5.3.1. Experimental set up

Seeds of common reed (*P. australis*) and cattail (*T. latifolia*; obtained from Stauden-Stade, Borken-Marbeck, Germany) were germinated in a greenhouse. After germination (30 days), several seedlings (between one and three) of a given species were transferred to plastic pots (PVC pipes of height 30 cm and Ø 160 mm, lined with a mesh to keep sediment in while allowing water to flow through) filled with surface sediment that was taken from a permanently water-filled kettle hole near Müncheberg, Germany (52°28'7.82"N, 14°8'27.99"E). In total, 84 pots for each species were established. We placed seven pots within a tub (65 liter, height 33 cm, Ø 60 cm), giving 24 tubs in total (12 per species; see Figure S 3e). The tubs were filled with water, submerging the pots, with the water table established approximately 3 cm above the sediment. A pumping system ensured oxygenated water could flow through the sediments in the pots (pictures see Figure S 3a-c). The experiment started after three months of growth (from seedlings to fully grown plants) and acclimation time, and simulated a summer drought as expected to occur more often in the future (Briffa *et al.* 2009). The 24 tubs were divided into the following treatments (n = 6): *P. australis* Control (PC), *P. australis* Drought (PD), *T. latifolia* Control (TC), *T. latifolia* Drought (TD).

Oxygen and pH of the tub water for control and pH of the sediment mixed with distilled water for drought treatments were determined using an oxi- and pH meter, respectively (WTW Oxi 320, WTW pH 330; WTW GmbH, Weilheim, Germany). We also measured sediment moisture content in the drought treatments at 10-min intervals (ECH2O EC-5; EM50 Digital Datalogger; Decagon Devices, Pullman, WA, USA).

A light sensor (QSO-S PAR Photon Flux sensor; Decagon Devices) and two sensors for relative air humidity and air temperature (VP-3 sensor; Decagon Devices) were installed at 5 cm height above the sediment between the plants. In addition, we measured photosynthetic leaf gas exchange with a portable gas exchange system (GFS3000; Walz, Effeltrich, Germany) using the following setting to ensure equal conditions during measurements: Photosynthetic active radiation of 650 µmol $m^{-2} s^{-1}$; relative humidity of 50%; CO_2 at 390 ppm; air flow of 750 µmol s^{-1}; and cuvette temperature of 25°C. We measured two replicates of each treatment per day, so after 3 days all plants were measured once. We then took the 3-day mean of the six replicates per treatment. Harvesting led to fewer replicates (minimum n = 2) towards the end of the experiment.

5.3.2. Drought treatment, $^{13}CO_2$ pulse labeling and harvest

The plants were subjected to the treatments (control or drought) for 1 month. Such short-term droughts have the highest occurrence in Europe and are more prevalent than longer-term droughts with > 3 months duration (Sheffield & Wood 2008). For the drought-exposed plants, the water was removed from the tubs and no additional water was added over the course of the experiment. In the control plants, the water table was kept 3 cm above the sediment (pictures see Figure S 3a, b).

We performed two $^{13}CO_2$ pulse labeling events, one for each species, for *P. australis* 12 days after the onset of drought (= Drought Day (DD) 12) and for *T. latifolia* on DD 16. The difference in labeling timing was due to the fact that several hours of sunshine were required during labeling and not all plants could be treated on the same day. This design was chosen, as the main point of our experiment was to compare the drought treatments for a given species and to obtain statistically robust results for the drought effects. A gas-tight chamber was placed on each tub and sealed (pictures see Figure S 3d). Then the plants were pulse labeled with $^{13}CO_2$ produced by adding 4 ml 80% H_3PO_4 to 0.8 g 99% ^{13}C-enriched sodium bicarbonate (Cambridge Isotope Laboratories, Andover, MA, USA). CO_2 concentrations were not controlled during the labeling. Fans inside the chambers dispersed the generated gas. The chambers were removed after 8 h of $^{13}CO_2$ fumigation. To minimize potential effects of back diffusion of $^{13}CO_2$ from the sediments and subsequent photosynthetic uptake, we ventilated the greenhouse with fans and open windows for 2 h after labeling. The fact that the pre-labeling isotopic signature of *T. latifolia* was not influenced by the labeling of *P. australis* 4 days before (and both species were located very close to each other), indicates that neither rest-label diffusion nor respiratory release of $^{13}CO_2$ had a significant influence on the carbon isotope signature of plant organic matter.

Over the month-long experimental drought, we harvested plants seven times (three samplings before the $^{13}CO_2$ labeling and four after). Sediment and plants were harvested on drought day (DD) 0, 4, 9, 13, 15, 18, and 26 for *P. australis* and on DD 0, 5, 14, 17, 19, 24, and 31 for *T. latifolia*. Sediment samples were taken with a spatula from depths between 5 and 25 cm close to the roots. Samples from five locations were bulked to one sediment sample. Plant samples consisted of leaves, stems, and roots. Sediment particles were washed from roots. We determined the fresh weight of the plant biomass and then stored the samples at -80°C. Dry weight of the plant biomass was determined by drying samples at 60°C in an oven to a constant mass. During each sampling, one pot per tub was harvested, resulting in six replicates for each species from the control and drought treatment at each time point.

Water content (%) in the plant tissues (leaves, stems, and roots) was calculated using the difference between fresh weight (FW) and dry weight (DW) of samples related to the FW.

5.3.3. Isotopic analysis in plant organic matter and in phospholipid-derived fatty acids

The carbon isotope composition ($\delta^{13}C$) was measured in the total organic matter and the water-soluble organic matter of different plant tissues (leaves, stems, and roots) as well as for sediment-extracted microbial phospholipid-derived fatty acids (PLFAs).

For $\delta^{13}C$ measurements of bulk plant tissues, the samples were dried for 48 h at 60°C and homogenized. The water-soluble organic matter – consisting mainly of sugars, polyols, organic acids, and amino acids (Brandes *et al.* 2006) – was obtained according to Gessler *et al.* (2009). In brief, 1 ml of demineralized cool (4°C) water was added to ca. 200 mg of a sample that was homogenized in liquid nitrogen. The mixture was incubated on a rotator for 1 h at 4°C and then heated at 99°C in a thermoshaker for 3 min. After centrifugation at 1400 rpm for 15 min, 50 µl of the supernatant were transferred to a tin capsule. The liquid was dried with a vacuum concentrator for 1 h at 30°C.

After preparation of the different plant samples, the isotopic composition was analyzed at the ZALF Isotope Core Facilities and the Ecosystem Fluxes Group of the Paul Scherrer Institute (PSI) by combusting the dried organic material in an elemental analyzer (Flash HT Elemental Analyzer, Thermo-Scientific; EA-1110; Carlo Erba Thermoquest, Milan, Italy) coupled to an Isotopic Ratio Mass Spectrometer (Delta V Advantage IRMS, Thermo-Scientific; Delta S). The isotopic values are expressed in delta notation (in ‰ units), relative to VPDB (Vienna Pee Dee Belemnite) and calibration was to IAEA-CH-6 (sucrose) and USGS40 (L-glutamic acid). Analysis of internal laboratory standards ensured that the estimates of the organic isotopic values were precise to within 0.1‰.

We computed the mean residence time (MRT) of total organic matter in leaves. MRT corresponds to the carbon stock to carbon flux ratio (Epron *et al.* 2012) and was calculated by fitting the following exponential decay function.

$$N(t) = N_0 e^{(-\lambda t)} \quad (2)$$

where *t* is the time in hours after 49lycol49ng; N_0 is the initial quantity of $\delta^{13}C$ at time t = 0 (^{13}C peak); λ is the decay constant; and N(t) is the quantity of ^{13}C after time t. The mean residence time (in hours) was then calculated as MRT = 1/ λ.

Excess ^{13}C ($mg^{13}C\ m^{-2}$) in leaves, stems, and roots was calculated after Ruehr *et al.* (2009):

$$\frac{atom\%_{Sample} - atom\%_{NA}}{100} \times B \times \frac{\%C}{100} \quad (3)$$

where $atom\%_{Sample}$ is the atom% ^{13}C of the sample after $^{13}CO_2$ labeling; $atom\%_{NA}$ is the atom% of the last sample taken before labeling (representing the natural abundance of ^{13}C); B is the DW of leaf, stem, or root biomass per ground area ($mg\ m^{-2}$); and %C is the percentage of carbon in the sample.

For phospholipid-derived fatty acid (PLFA) extraction, frozen sediment samples were freeze-dried and 1 g of dry sediment was extracted with a modified one-phase Bligh/Dyer method (Frostegård *et al.* 1991; Steger *et al.* 2015). The lipids were then separated into different lipid classes with increasing polarity (neutral, 50lycol-, and phospholipids) using solid phase extraction with silicic acid columns (BondElut LRC-Si; Agilent Technologies Inc., Santa Clara, CA, USA). The fatty acids 19:0 and 21:0 were added to the samples as an internal standard. The extracted PLFA samples were dried and stored at -20°C until further analysis. Quantification and identification of PLFAs were performed on a gas chromatograph (Steger *et al.* 2011). We used standard nomenclature to refer to the PLFAs (Boschker *et al.* 2005; Kaur *et al.* 2005; Denef *et al.* 2009; Steger *et al.* 2011). The stable carbon isotopic composition of the individual PLFAs was determined on a Thermo-Scientific GC/C-IRMS system (Thermo Trace GC Ultra gas chromatograph coupled to a Delta V Advantage IRMS) at the UC Davis Stable Isotope Facility. PLFA $\delta^{13}C$ data were corrected for the addition of the methyl group and were calibrated by our own internal and external fatty acid methyl ester (FAME) standards. Stable carbon isotope ratios are reported on the VPDB scale.

The ^{13}C uptake into the microbial PLFA biomass is expressed as excess ^{13}C PLFA (µg ^{13}C kg^{-1}). The excess ^{13}C PLFA represents the total amount of ^{13}C in the microbial PLFAs per kilogram sediment and is calculated after Fuchslueger *et al.* (2014):

$$\frac{(\text{atom\%}_{Sample} - \text{atom\%}_{NA}) \times \text{Biomass}}{100} \times 1000\ (g) \qquad (4)$$

Where $\text{atom\%}_{\text{Sample}}$ is the ^{13}C atom % of the labeled PLFA sample; $\text{atom\%}_{\text{NA}}$ is the atom% of the last PLFA sample taken before labeling (representing the natural abundance of ^{13}C); and Biomass is the PLFA biomass (µg C).

5.3.4. Concentration of non-structural carbon compounds

Dried leaves, stems, and roots of both species were ground to a homogenous powder. Water-soluble carbon compounds were extracted according to Peuke *et al.* (2015) with deionized water. After agitating and heating, the samples were centrifuged and the supernatant was frozen at -18°C until further analysis. For starch extraction, the pellets from the sugar extraction were washed and then dried at 60°C. 1 ml enzymatic solution (9.21 mg amyloglucosidase in 25 ml acetate buffer, pH 4.8) was added to the samples for starch digestion. Aliquots of the extracts from water-soluble sugar extraction and starch digestion were injected into a Dionex DX 600 HPLC system (Thermo Scientific, Dionex, Idstein, Germany) with a 4 × 250 mm Carbo Pac PA 1 column (Dionex) with 100 mM NaOH as mobile phase. Detection and quantification were performed with a pulsed amperometric detector (Dionex ED 50 electrochemical detector). From the amount of glucose in the starch extracts, the

starch concentration was calculated according to Janzen *et al.* (1968). NSC is the sum of glucose, fructose, sucrose, and starch. Values are given in mg per g dry sample.

5.3.5. Statistical analysis

Treatment differences in environmental and isotopic values were tested using repeated measures ANOVA followed by a Tukey HSD test. Results were compared against the main effects time and treatment (comparing PC with PD and TC with TD) in a two-way ANOVA. If interactions were significant, only these significances were taken into account. We applied a Tukey post hoc test when significances were given. With the large differences through labeling and treatment, most of our results did not strictly have a normal distribution and/or homogeneity of variance, which was tested using a Shapiro-Wilk test (for normality) and Levene's test (for homogeneity). This is, however, the case for most ecological multivariate data sets, and moderate non-normal distributions should not have large effects on the ANOVA (Anderson 2001). Statistical analysis was carried out in R version 2.15.1 (R Development Core Team 2008). Differences in MRT between treatments were assessed by a Student's t-test.

5.4. Results

5.4.1. Environmental parameters

Sediments of controls remained submerged under water, thus being saturated over the whole course of the experiment. Sediment moisture in the drought treatments gradually decreased from 0.46 ± 0.06 m^3 m^{-3} (± SD) volumetric water content (VWC) at the beginning of the experiment and reached 0.07 ± 0.01 m^3 m^{-3} after 18 days of drought (Figure 5-1a). The low VWC values at the end of the experiment could be a result of the sensors being exposed to air. When the sediment dried out, cracks appeared on the sediment surface, which might have resulted in the sensors no longer being completely covered. A t-test confirmed that the decrease in sediment moisture was not significantly different (P = 0.48) between *P. australis* and *T. latifolia.* Mean daily air temperatures during the experiment varied between 21 and 28°C, mean daily relative humidity between 41 and 70% (Figure 5-1b), and daily photosynthetically active radiation (PAR) between 57 and 248 µmol m^{-2} s^{-1} during the light period (data not shown).

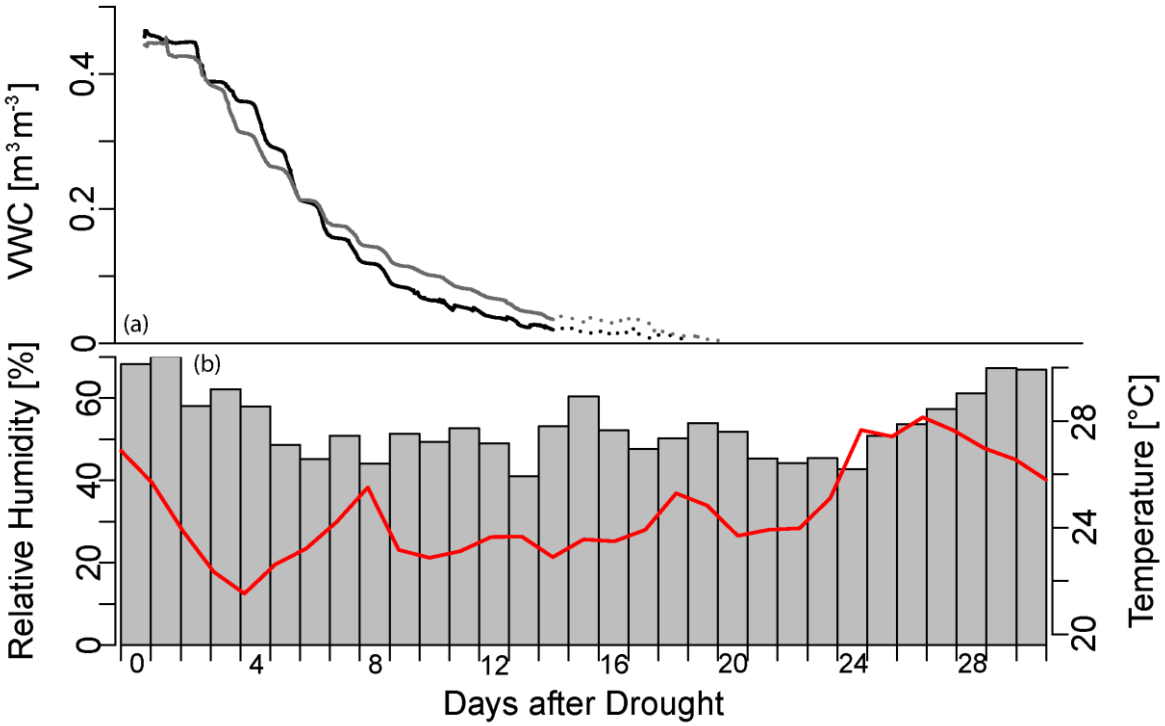

Figure 5-1: Environmental parameters during the course of the experiment. (a) Sediment moisture as volumetric water content (VWC; m^3 m^{-3}) for *P. australis* (black) and *T. latifolia* (gray) for the drought treatment. Values are means (n = 2 to 20) measured at 10-min intervals. Dotted lines at the end of the moisture measurements (last 6 days) indicate potentially imprecise measurement due to sediment crack formation. (b) Relative humidity (%; left axis, bars) and temperature (°C; right axis red line) during the experiment. Values represent daily means of 30-min interval measurements.

The pH in the controls (PC and TC) remained stable at around 8.5 (Table S 3). In the drought treatments, pH decreased over time and reached values of approx. 7.0 for both species 23 days after the onset of drought (DD 23). The oxygen concentration in the tub water of the control treatments did not decrease below 6.1 mg l^{-1} and displayed no clear temporal pattern (Table S 3).

5.4.2. Plant biomass

There was a slight increase in the shoot biomass over the experimental period for both species in the control treatments (Figure 5-2a). Shoot DW of *T. latifolia* was significantly lower in the drought treatment compared to the control ($P < 0.01$). In roots, the DW of *P. australis* was significantly lower in the drought treatment compared to the control ($P < 0.01$; Figure 5-2b). The average root:shoot ratio amounted to 3.4 and 1.4 for *P. autralis* and *T. latifolia* and was not significantly affected by drought for both species (Figure 5-2c).

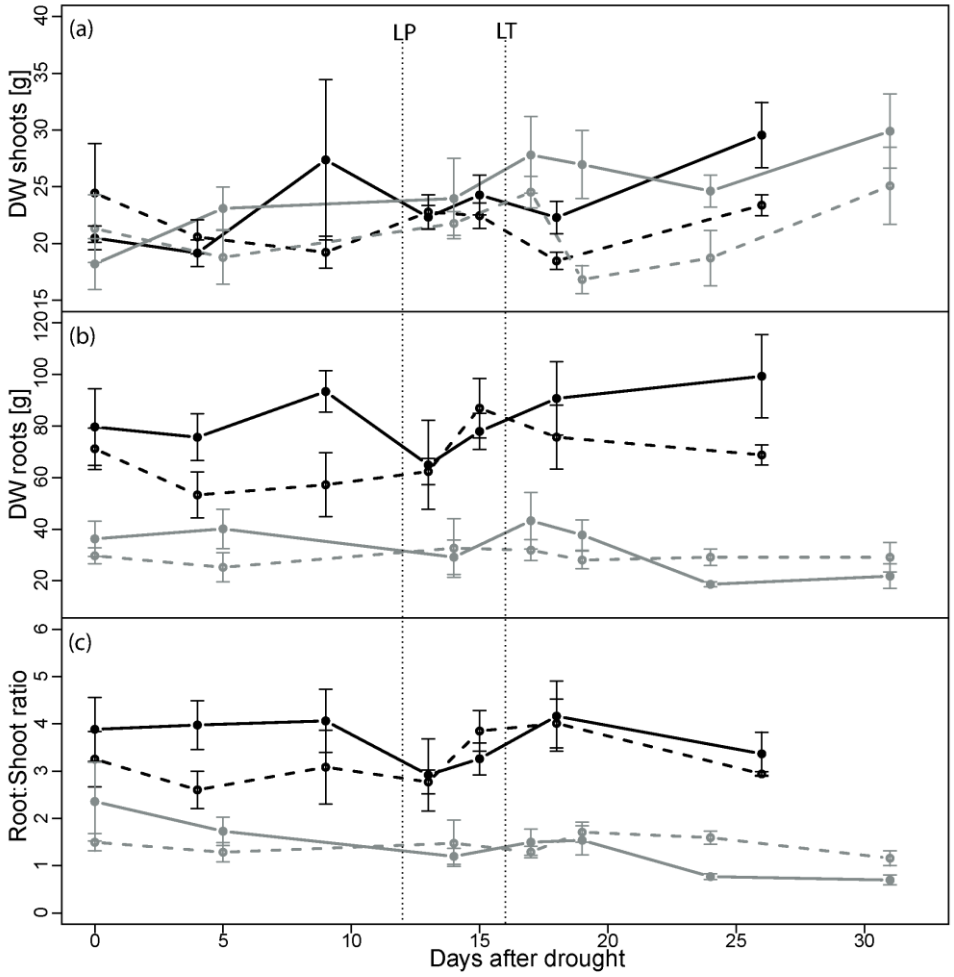

Figure 5-2: Plant biomass parameters over the course of the experiment. Dry weight (DW) of shoots (a) and roots (b) as well as the root:shoot ratio (c) for *P. australis* (black) and *T. latifolia* (gray) for control (solid lines) and drought (dotted lines) are displayed. Values are means (n = 6) and error bars indicate SE. Note that the y-axis scales are different for (a) and (b). LP and LT: time of ^{13}C pulse labeling for *P. australis* and *T. latifolia*, respectively.

There was no significant difference in the tissue water content over time in the control for leaves in both species (Figure S 4a). For stems, water content on DD 31 was significantly different to DD 0 ($P < 0.05$) in the control for *T. latifolia* (Figure S 4b). For roots, there was a significant increase over time in both species ($P < 0.05$) in the control (Figure S 4c). In both species, drought lowered the water content in all tissues. In *P. australis*, water contents in the different tissues were reduced significantly from DD 15 ($P < 0.05$) and in *T. latifolia* from DD 24 ($P < 0.05$).

5.4.3. Photosynthesis and stomatal conductance

Photosynthesis (A) and stomatal conductance (g_s) did not change significantly over time in the controls for both species. For the controls of *P. australis* and *T. latifolia*, A ranged from 5.0 to 7.3 µmol $m^{-2} s^{-1}$ and from 6.9 to 9.8 µmol $m^{-2} s^{-1}$, respectively. Stomatal conductance values ranged from 57.5 to 125.4 mmol $m^{-2} s^{-1}$ and from 82.6 to 167.2 mmol $m^{-2} s^{-1}$, respectively. With the drought progressing, there was a significant decrease in A and g_s, with both species acting similar but with a stronger change in *T. latifolia*. In *P. australis*, A and g_s decreased significantly in the drought treatment, starting from DD 12 ($P < 0.05$) when A was 2.0 µmol $m^{-2} s^{-1}$. The lowest A for PD was reached on DD 15 amounting to 0.3 µmol $m^{-2} s^{-1}$. In *T. latifolia* the difference in A and g_s between control and drought became significant on DD 15 ($P < 0.05$) when A in the drought treatment was 2.0 µmol $m^{-2} s^{-1}$. The lowest A for TD was reached on DD 22 amounting to 0.1 µmol $m^{-2} s^{-1}$ (Figure 5-3).

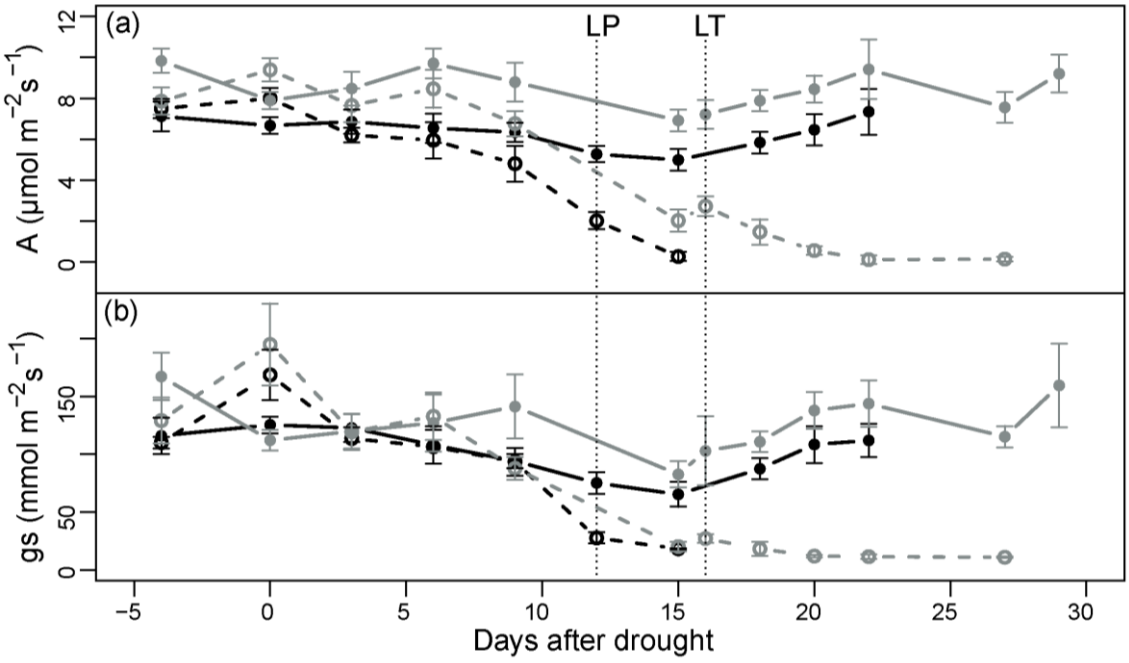

Figure 5-3: Gas exchange during the course of the experiment. (a) Photosynthesis (A; µmol m^{-2} s^{-1}) and (b) stomatal conductance (g_s; mmol m^{-2} s^{-1}) over time for *P. australis* (black) and *T. latifolia* (gray) for control (solid lines) and drought (dotted lines). Values are 3- to 1-day means (n = 6 at beginning of experiment; reduced to 2 at the end). Error bars indicate SE. LP and LT: time of ^{13}C pulse labeling for *P. australis* and *T. latifolia*, respectively.

5.4.4. Non-structural carbon compounds

In *P. australis*, glucose and fructose concentrations in leaves showed similar trends over time, with the control being significantly different from the drought treatment on DD 13 and 26 ($P < 0.05$). In PC there was no significant change in glucose and fructose concentrations over time, and average values amounted to 15.6 and 17.5 mg g^{-1}, respectively (Figure 5-4a). In PD, glucose and fructose concentrations increased significantly with progressing drought ($P < 0.05$) reaching maximum values of 31.4 and 36.2 mg g^{-1}, respectively, on DD 26 (Figure 5-4b). For sucrose, starch, and total NSC, PC and PD were not significantly different.

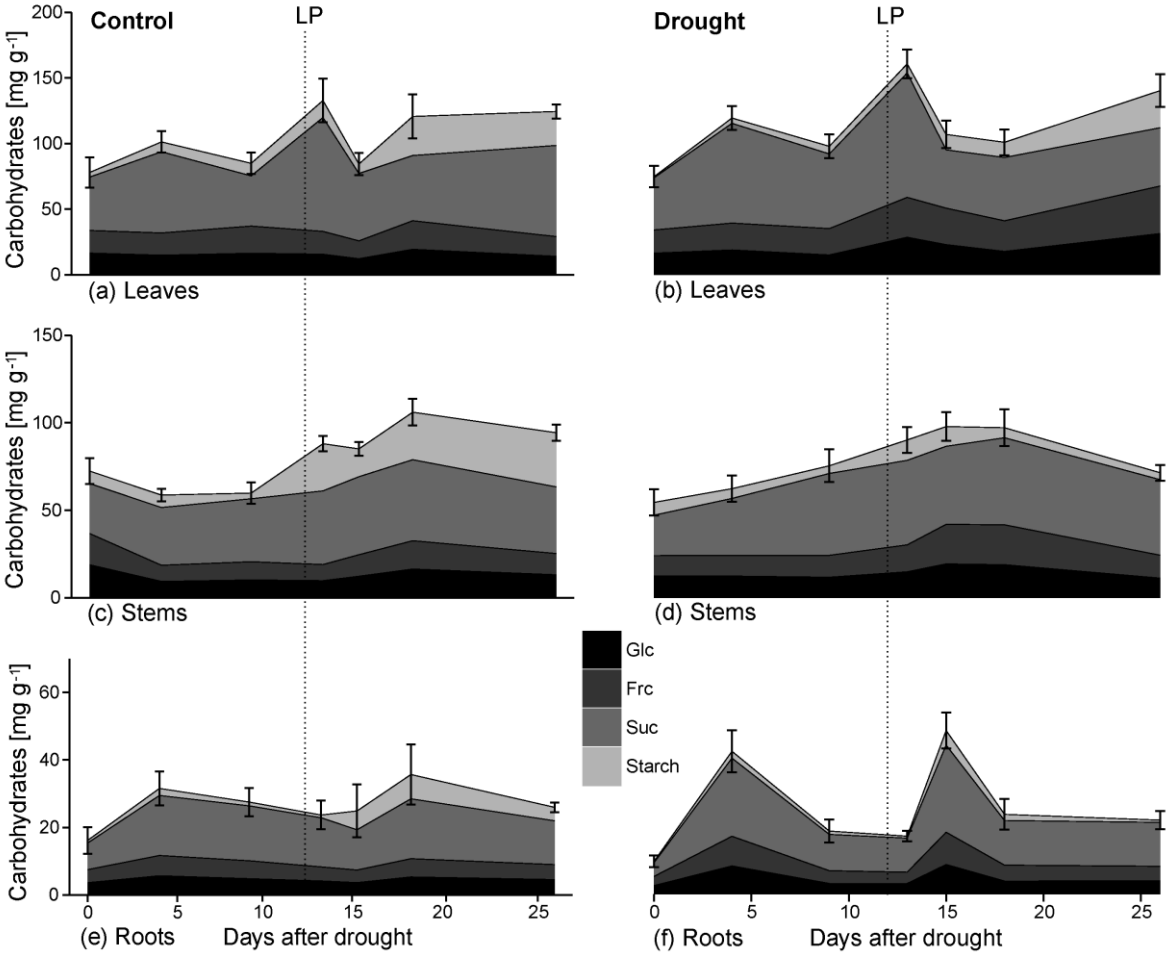

Figure 5-4: Temporal course of non-structural carbon compounds (NSC) in control and drought for different compartments (leaves, stems and roots) of *P. australis* (mg g^{-1}). The graphs are stacked area graphs and the different gray tones indicate the different compounds. The whole area of the graphs represents total NSC (Glc + Frc + Suc + Starch). Values are means (n = 6). Error bars indicate SE for total NSC. SE of single compounds have been omitted for clarity. LP is the time of ^{13}C pulse labeling for *P. australis*.

In *T. latifolia* leaves, glucose only changed slightly over time, with no significant difference between TC and TD, while fructose was significantly lower in the drought treatment ($P < 0.001$). Sucrose was significantly higher in TC compared to TD by 39.7 and 68.7 mg g^{-1} on DD 19 and 24 ($P < 0.05$), respectively (Figure 5-5a, b). For starch and total NSC in leaves, concentrations did not change significantly over time in TD. In the control, starch had a significant peak on DD 5, with 14.5 mg g^{-1} and was thus significantly higher than TD ($P < 0.01$). For total NSC in leaves, TC exceeded TD on DD 24 ($P < 0.001$) by 46.0 mg g^{-1} and DD 0 was significantly different to DD 5, 19, and 24 ($P < 0.05$). In summary, leaf NSC increased slightly in the control of *T. latifolia* over time, while in contrast a decrease was observed in the drought treatment.

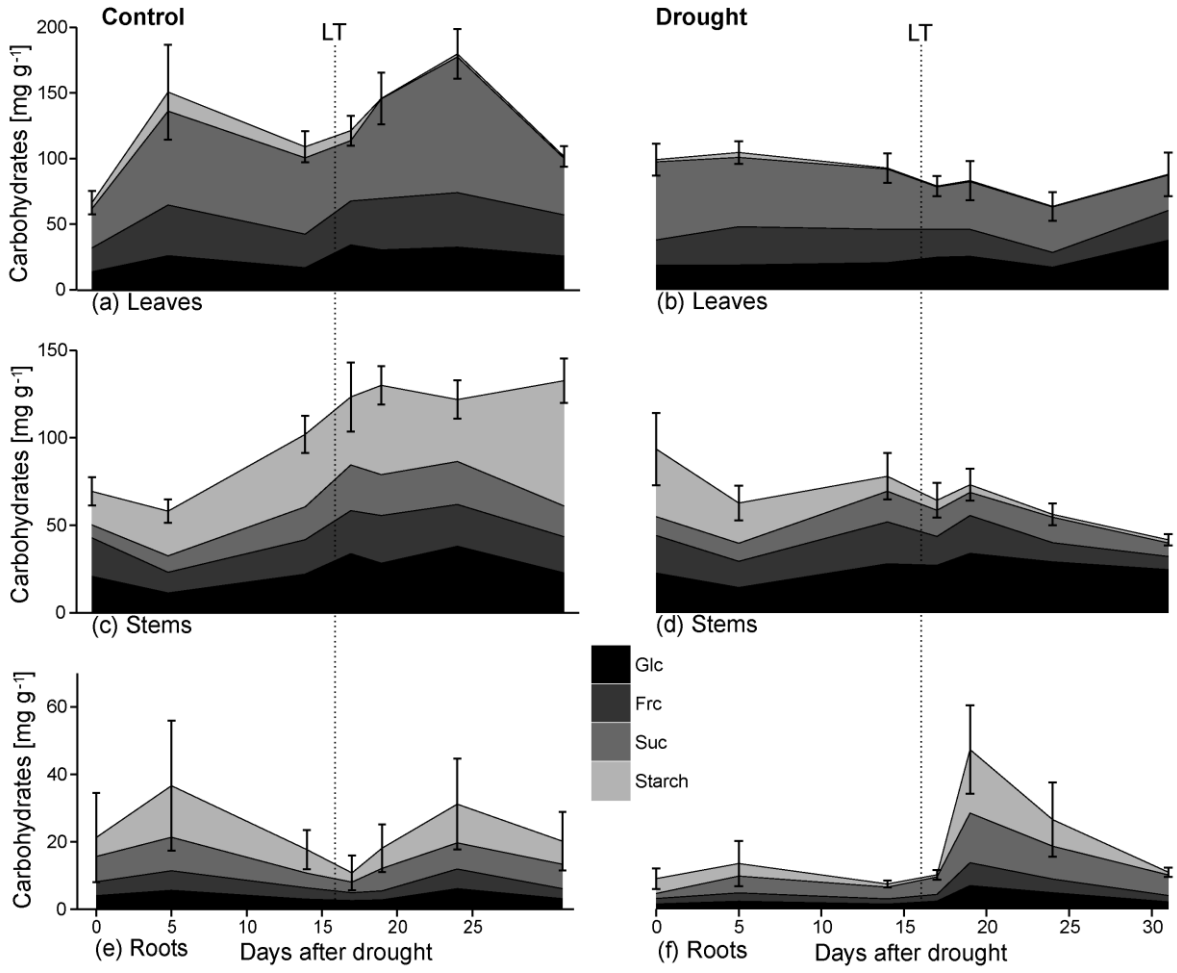

Figure 5-5: Temporal course of non-structural carbon compounds (NSC) in control and drought for different compartments (leaves, stems and roots) of *T. latifolia* (mg g^{-1}). The graphs are stacked area graphs and the different gray tones indicate the different compounds. The whole area of the graphs represents total NSC (Glc + Frc + Suc + Starch). Values are means (n = 6). Error bars indicate SE for total NSC. SE of single compounds have been omitted for clarity. LT is the time of ^{13}C pulse labeling for *T. latifolia*.

In *P. australis* stems, glucose and sucrose were not significantly different between C and D (Figure 5-4c, d). Fructose concentrations showed a slight peak under drought on DD 15 and 18, being significantly different ($P < 0.05$) to the initial values and the values at the end of the drought period, and thus caused a significant difference on DD15 ($P < 0.05$) between C and D. Starch was significantly increased in the control compared to the drought treatment on DD 18 and 26 ($P < 0.001$) by 21.4 and 27.0 mg g^{-1}, respectively, at the end of the treatment. Total NSC concentrations were not significantly different between treatments. In both treatments, NSC increased over time, reaching maximum values of 106.0 and 97.8 mg g^{-1} for PC and PD, respectively.

In *T. latifolia* stems, glucose, fructose, and sucrose were not significantly different between C and D (Figure 5-5c, d). However, starch and the total NSC concentration in stems were significantly ($P < 0.05$) different between TC and TD after some time of drought exposure (DD 19, 24, and 31 for total NSC; DD 14, 17, 19, 24, and 31 for starch). Thus, stems of control plants accumulated additional starch during the course of the experiment, while drought-exposed individuals almost totally consumed the starch present at the beginning.

In *P. australis* roots, glucose, fructose, and sucrose concentrations were significantly different between PC and PD on DD 15 ($P < 0.05$) in which all three compounds showed a strong peak (glucose: 8.8 mg g^{-1}, fructose: 9.6 mg g^{-1}, sucrose: 25.9 mg g^{-1}) in the drought treatment (Figure 5-4e, f). Over time, the concentrations of the three free sugars also varied significantly ($P < 0.05$) showing two local maxima. Starch and total NSC concentrations were, however, not significantly different between PC and PD. There was, in general, some oscillation in the contents of the free sugars over time in both treatments, and the variation was more pronounced under drought.

Such oscillations were also detected in the roots of *T. latifolia* in both treatments (Figure 5-5e, f), and there was no significant difference between C and D, neither for single compounds nor for total NSC. The NSC concentrations in the different compartments decreased in the order leaves > stems > roots.

5.4.5. Enrichment of ^{13}C in plant compartments and in water-soluble organic matter

The $\delta^{13}C$ values in the total organic matter (OM), in the water-soluble organic matter fraction, as well as ^{13}C excess in total organic matter peaked in leaves of both species in C and D on the first day after labeling. The ^{13}C increase upon labeling was significant ($P < 0.001$) for both total and water-soluble OM. Thereafter, the label decreased until day 14 after labeling (Figure 5-6).

The $\delta^{13}C$ in total OM and in the soluble OM fraction in *P. australis* leaves was not significantly different between PC and PD. Moreover, the scaling of the ^{13}C label incorporated to the total leaf biomass per m^2 (^{13}C excess) also revealed no difference between PC and PD (Figure 5-6a, b).

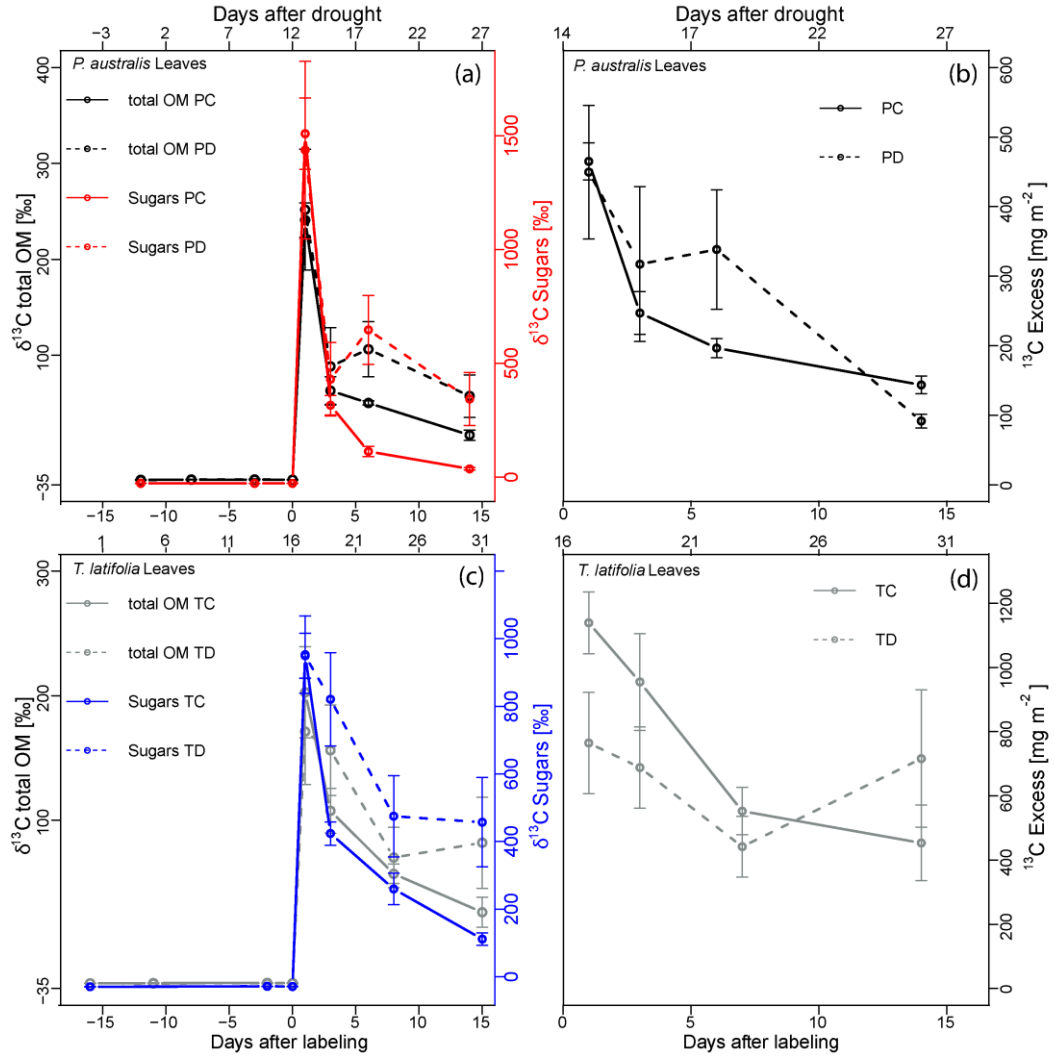

Figure 5-6: Temporal course of the ^{13}C label in leaves. (a) and (c) $\delta^{13}C$ for total organic matter and water-soluble organic matter (sugars; ‰) for *P. australis* and *T. latifolia*, respectively. Black and red colors indicate total organic matter and water-soluble organic matter (sugars) for *P. australis* respectively. Gray and blue colors indicate total organic matter and water-soluble organic matter (sugars) for *T. latifolia*, respectively. Solid lines indicate controls, dotted lines the drought treatments. (b) and (d) ^{13}C excess (mg m^{-2}) for leaf total organic matter in *P. australis* and *T. latifolia*. The lower x-axes show days after labeling, the upper x-axes days after drought. Values are means (n = 6). Error bars indicate SE. The data point at the labeling day is the mean of the natural abundance values measured. PC = *P. australis* control, PD = *P. australis* drought, TC = *T. latifolia* control, TD = *T. latifolia* drought. Note that axis scales are different for *P. australis* and *T. latifolia*.

For T. *latifolia*, $\delta^{13}C$ in total OM of leaves and leaf ^{13}C excess were not significantly different between TC and TD after labeling. $\Delta^{13}C$ in the water-soluble organic matter, however, was higher in TD compared to TC (especially on DD 19; $P < 0.05$). While the peak on day 0 after labeling was comparable between TC and TD, the label decreased more strongly in the subsequent days in the controls (Figure 5-6c, d).

The mean residence time (MRT) of total leaf OM was not significantly different between treatments for both species. For *P. australis* it amounted to 1.7 ± 0.3 days and 3.3 ± 1.7 days in C and D, respectively, and for *T. latifolia* to 1.9 ± 0.4 days and 2.5 ± 0.9 days.

In the stems of *P. australis*, $\delta^{13}C$ in the total organic matter as well as in the water-soluble organic matter fraction peaked for both PC and PD on the first day after labeling (Figure 5-7a). The water-soluble OM – representative mainly for sugars and thus the fast turnover carbon pool – also displayed highest $\delta^{13}C$ values on day 1 after labeling in *T. latifolia,* but the ^{13}C tracer in total OM peaked 3 days after labeling (Figure 5-7c). The ^{13}C increase after labeling was significant ($P < 0.001$) for both total and water-soluble OM.

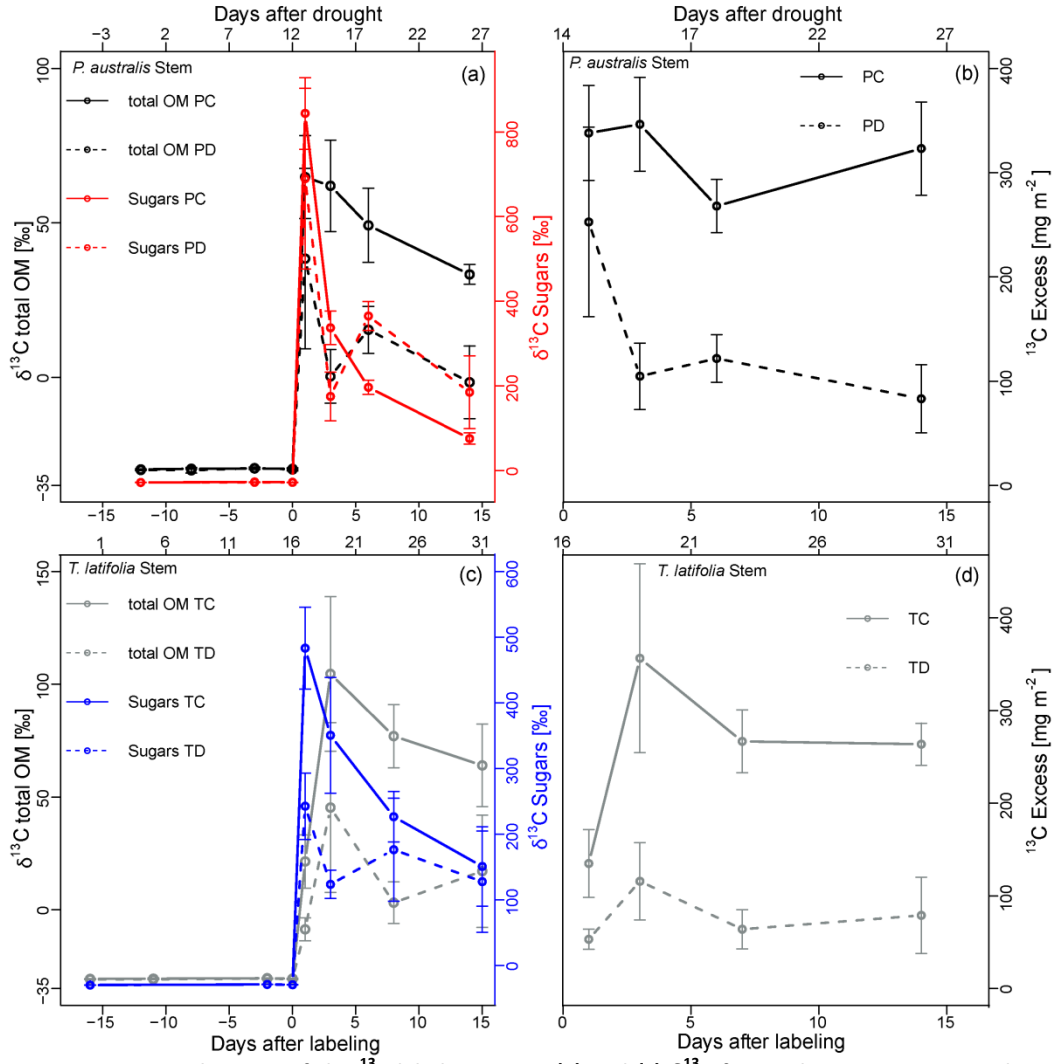

Figure 5-7: Temporal course of the ^{13}C label in stems. (a) and (c) $\delta^{13}C$ for total organic matter and water-soluble organic matter (sugars; ‰) for *P. australis* and *T. latifolia*, respectively. Black and red colors indicate total organic matter and water-soluble organic matter (sugars) for *P. australis* respectively. Gray and blue colors indicate total organic matter and water-soluble organic matter (sugars) for *T. latifolia*, respectively. Solid lines indicate controls, dotted lines the drought treatments. (b) and (d) ^{13}C excess (mg m^{-2}) for stem total organic matter in *P. australis* and *T. latifolia*. The lower x-axes show days after labeling, the upper x-axes days after drought. Values are means (n = 6). Error bars indicate SE. The data point at the labeling day is the mean of the natural abundance values measured. PC = *P. australis* control, PD = *P. australis* drought, TC = *T. latifolia* control. TD = *T. latifolia* drought. Note that axis scales are different for *P. australis* and *T. latifolia*.

Significant differences between C and D for $\delta^{13}C$ in total OM were found in *P. australis* (DD 15; $P < 0.05$) and in the soluble OM fraction in *T. latifolia* ($P < 0.01$). Moreover, scaling of the ^{13}C to the total stem biomass per m^2 (^{13}C excess) revealed a significant difference (with higher label amounts in the controls) between C and D for both species ($P < 0.001$; Figure 5-7b, d).

The $\delta^{13}C$ in total OM of *P. australis* roots peaked 3 days after pulse labeling in PC and stayed approximately constant thereafter. In PD, the peak was delayed and visible 6 days after label application. $\Delta^{13}C$ in the water-soluble OM fraction showed a more scattered pattern, with highest values on days 1 and 3 after labeling in PC and on days 1 and 6 in PD (Figure 5-8a). In roots of *T. latifolia*, $\delta^{13}C$ in total OM increased in TC until the end of the measurement period. In TD, in contrast, the highest $\delta^{13}C$ value was observed on day 3 after labeling. In the water-soluble OM fraction highest $\delta^{13}C$ values were observed 1 and 3 (TC) and 3 days (TD) after label application (Figure 5-8c).

Significant differences between C and D for $\delta^{13}C$ in total OM were found in *P. australis* and *T. latifolia* (DD 26 for P, DD 31 for T; $P < 0.01$) and in the water-soluble OM fraction in *T. latifolia* ($P < 0.05$). Moreover, scaling of the ^{13}C to the total root biomass per m^2 (^{13}C excess) revealed a significant difference, with higher label amounts in the control between PC and PD ($P < 0.001$; Figure 5-8b, d).

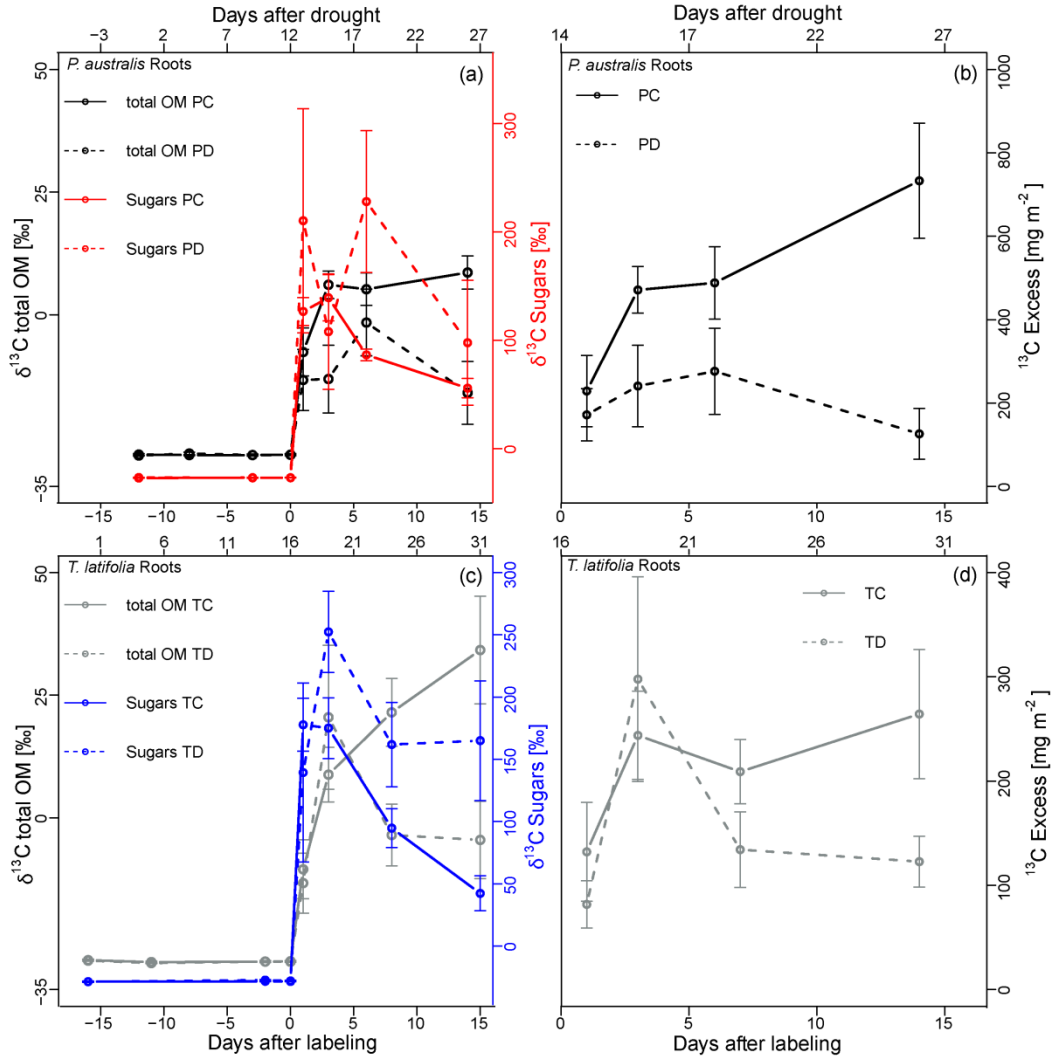

Figure 5-8: Temporal course of the ^{13}C label in roots. (a) and (c) $\delta^{13}C$ for total organic matter and water-soluble organic matter (sugars; ‰) for *P. australis* and *T. latifolia*, respectively. Black and red colors indicate total organic matter and water-soluble organic matter (sugars) for *P. australis* respectively. Gray and blue colors indicate total organic matter and water-soluble organic matter (sugars) for *T. latifolia*, respectively. Solid lines indicate controls, dotted lines the drought treatments. (b) and (d) ^{13}C excess (mg m^{-2}) for root total organic matter in *P. australis* and *T. latifolia*. The lower x-axes show days after labeling, the upper x-axes days after drought. Values are means (n = 6). Error bars indicate SE. The data point at the labeling day is the mean of the natural abundance values measured. PC = *P. australis* control, PD = *P. australis* drought, TC = *T. latifolia* control, TD = *T. latifolia* drought. Note that axis scales are different for *P. australis* and *T. latifolia*.

5.4.6. Enrichment of ^{13}C in phospholipid-derived fatty acids (PLFAs)

We analyzed 11 PLFAs to evaluate different groups of microorganisms (see Table S 4 for a list of used PLFAs). The remaining PLFAs that had been extracted did not yield sufficient material for analysis (i.e., they were below the detection limit).

In general, the ^{13}C excess was lower in the drought treatments, compared to the controls for both species, but label was still incorporated into bacterial and fungal PLFAs indicated by ^{13}C excess values > 0 (Figure 5-9). In addition, the peak label in PLFAs was observed later in D compared to C. In *P. australis* sediments, four out of 11 PLFAs peaked on day 3 after labeling in controls, whereas in PD peaks occurred only after 6 days. In *T. latifolia* seven out of 11 PLFAs showed highest ^{13}C excess on day 1 after labeling in the controls, but only one out of 11 in the drought treatment. The highest ^{13}C excess was observed in all plants and treatments in the unspecific c16:0, common to bacteria and algae. The ^{13}C excess, however, decreased significantly upon drought. In the controls, c18:2ω6, a marker for fungal biomass, was also highly labeled in sediment microorganisms of both species. In both species, the label incorporation was significantly lower for this fungal biomarker upon drought ($P < 0.01$). i16:0, a PLFA marker for gram-positive bacteria, was labeled more strongly in D when compared to C in both species, with significantly higher values on days 3 and 6 after labeling for *P. australis* and on day 3 for *T. latifolia* ($P < 0.01$). In contrast, for the gram-negative marker PLFA cy19:0, values of D were significantly lower than the C values. Heterotrophic bacteria marker PLFAs (a15:0, i15:0) rather peaked at the end of the experiment in the controls for both species.

For *P. australis* sediment microorganisms, the ^{13}C excess was additionally lower in the drought treatment compared to the control over all sampling time points for c16:1ω9c/7c, c18:0, and c18:1ω9c. For *T. latifolia* c16:1ω9c/7c, c18:0, c18:1ω9c, c18:1ω9t/7c, and a15:0 were also lower over the whole sampling period under D compared to C.

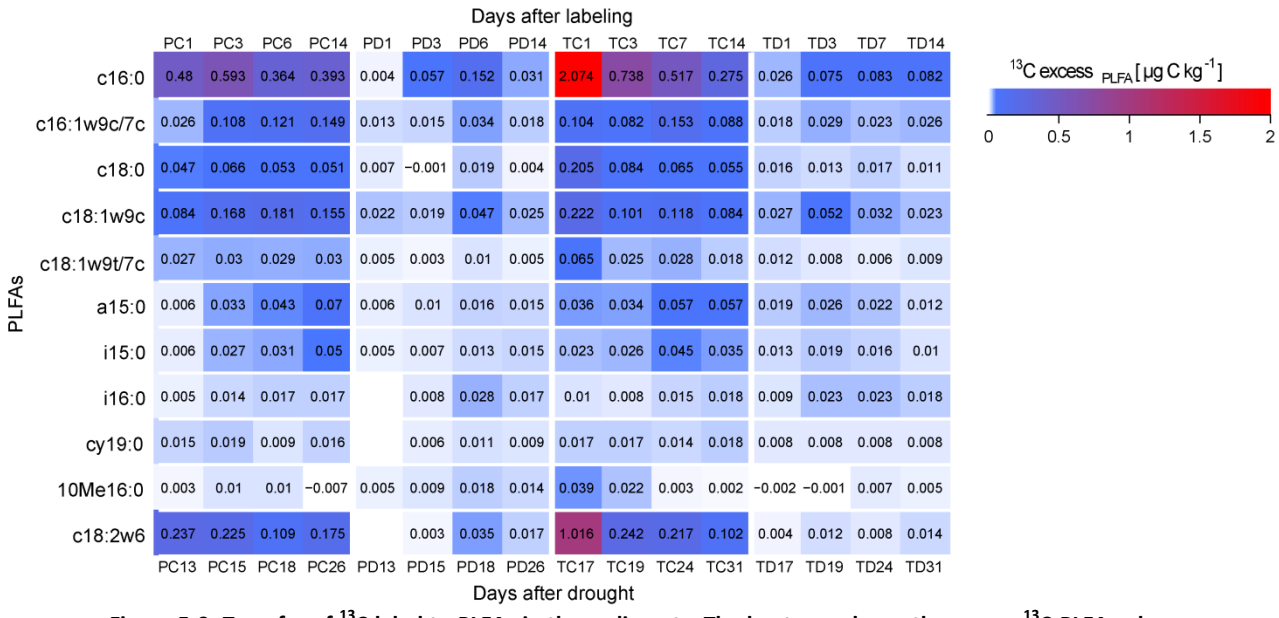

Figure 5-9: Transfer of ^{13}C label to PLFAs in the sediments. The heatmap shows the excess ^{13}C-PLFA values (µg C kg^{-1}) during the experiment for *P. australis* (P) and *T. latifolia* (T) in the control (C) and the drought treatment (D) for different PLFAs. The upper x-axis displays days after labeling and the lower x-axis days after the onset of the drought treatment. Values are means from three replicates of three pooled samples.

5.5. Discussion

From terrestrial plants it is well known that restricted water supply not only reduces plant photosynthesis but also impairs the coupling between photosynthetic carbon assimilation and belowground sink tissues of plants, as well as the transfer to the rhizosphere (Ruehr *et al.* 2009; Burri *et al.* 2014; Hasibeder *et al.* 2015; von Rein *et al.* 2016). It is assumed that storage reserves can be used for energy demanding belowground processes when recent assimilate supply is blocked, but there are strong indications that this carbon source is by far not sufficient to compensate for the new assimilates (Hogberg *et al.* 2001; Pena *et al.* 2010). To assess whether such uncoupling also occurs in aquatic/semi-aquatic ecosystems, we designed our experiments in a mesocosm with two emergent macrophyte species (*P. australis* and *T. latifolia*).

5.5.1. Drought does not alter assimilate transport velocity to roots but causes new assimilates to be used for root osmoregulation

Total leaf non-structural carbon (NSC) concentrations showed, in general, similar patterns between control and drought in *P. australis.* Glucose and fructose were increased – partially at the expense of sucrose – at the end of the drought period. We thus see no clear indication of an accumulation of sugars to increase leaf osmolality in response to drought. This finding is in contrast to results for common reed under increased salinity (Hartzendorf & Rolletschek 2001). We consequently assume that even though stomatal closure and reduction of assimilation occurred, the drought stress applied here was not severe enough to trigger an increase in leaf sugar concentration.

An increase in leaf sugar concentrations is often seen as a sign of reduced sink tissue activity at the beginning of a drought period when photosynthesis increases the sink tissue demand (McDowell *et al.* 2011). Reduced activity and thus carbon demand of sink tissues would also increase the NSC concentrations in stems and roots. Since we did not observe a clear change in leaf or in sink tissue NSC, a sink driven control of the carbon balance in *P. australis*, as recently suggested as a general mechanism in plants (Körner 2015), under drought seems unlikely. In *T. latifolia*, sucrose and fructose concentrations of leaves, as well as starch concentrations in the stems were lower under drought compared to control conditions. This might indicate that in this species, the reduction of photosynthesis determined the supply of new assimilates for the sink tissues and thus finally sink activity, as depicted by Sala *et al.* (2012). Upon $^{13}CO_2$ exposure, $\delta^{13}C$ in total and water-soluble organic matter (OM) peaked in leaves of both species on day 1 after labeling and the highest values observed were comparable among treatments. However, the amount of recently assimilated carbon that was transported from leaves to other plant compartments was altered under drought conditions in both species. In stems, the amount of labeled carbon was in general reduced, while in the roots of both species, $\delta^{13}C$ after labeling differed between total OM and the water-soluble OM fraction: while

the maximum $\delta^{13}C$ in total OM was comparable or lower in the drought treatment compared to the control, the opposite was observed for water-soluble OM (Figure 5-6, Figure 5-7, Figure 5-8). This increase in accumulation of the ^{13}C labeled water-soluble OM fraction in the roots might point to the investment of this carbon in osmotic adjustment. This finding is in line with observations from Hasibeder *et al.* (2015), who found that species from an alpine meadow allocated recent assimilates preferentially to osmotically active compounds under reduced soil water availability and thus expanded the pool of labeled sugars. This hypothesis seems, however, to contradict our observation that neither the concentrations of sucrose nor of the monosaccharides glucose and fructose strongly increased upon drought in the roots (Figure 5-4, Figure 5-5). However, other osmolytes than sugars have been observed to be important in *P. australis*: Hartzendorf & Rolletschek (2001) showed that amino acids were more relevant than sugars for osmoprotection in *P. australis* roots, and Briens & Larher (1982) reported high levels of polyols under salt stress. The water-soluble organic matter fraction contains both compound classes and thus it seems likely that both species rely on such osmoprotectants in their roots, besides sugars.

We observed strong short-term variation in the concentrations of sugars and total NSC in the roots of both species. Such patterns were also present in the controls, but the amplitudes were higher under drought. Temporal variations might be related to periodic fine root growth reducing the sugar concentrations, but the stronger oscillation can also be a sign of active accumulation of sugars for osmotic adjustment. The root NSC patterns under drought can be seen as a subtle balance between supply, accumulation of sugars for osmotic adjustment, and consumption for respiration, root repair, and growth (Brunner *et al.* 2015). Both increases and decreases of sugar concentrations in roots have been observed under drought (Regier *et al.* 2009; Zang *et al.* 2014), pointing to different balances between supply, accumulation, and consumption. The oscillations observed in our study might be due to strong temporal shifts in NSC supply from the shoots in relation to demand for metabolic processes and accumulation for osmotic regulation – pointing to a stress-related disturbance of the regulation of NSC homoeostasis.

In contrast to studies with trees (e.g., Barthel *et al.*, 2011), we did not find a delay in the arrival of the label in stems or roots. In general, phloem transport can be delayed by strongly negative xylem water potential as observed under drought (Sala *et al.* 2010). Phloem and xylem are assumed to have a tight hydraulic connection (Savage *et al.* 2015) and thus phloem transport velocities might be reduced due to high solute concentrations (causing high viscosity) in the sieve tubes needed for osmotic adjustment when xylem water tension is high (Hölttä *et al.* 2009). Even though the total amount of ^{13}C that arrived in stem tissues was reduced, the transport velocity was not affected in the two species examined. We might assume that photosynthetic activity of the green stems of both species was partially responsible for this observation, as ^{13}C label is also directly incorporated into

stem organic matter. In addition, phloem transport velocity of the two fast-growing aquatic species might be generally higher than in slower-growing trees. We have to reject part of our hypothesis (1) and conclude that under the restricted sediment water availability, the speed of linkage between above- and belowground plant parts was not affected. Moreover, there was no straightforward reduction in carbon allocation within the plants: it is obvious that the structural carbon pools in stem tissues were supplied with less recent assimilates, as indicated by the reduction of ^{13}C in total organic matter but not in the water-soluble fraction (Figure 5-7), but in roots the situation was more complex. While the maximum of $\delta^{13}C$ detected in root total OM was not strongly different between drought and control in both species, the ^{13}C excess in *P. australis* was significantly lower under drought. Most probably, the sensitivity of a particular species together with the intensity and duration of drought plays an important role in the reduction of the amount of sugars transported in the phloem as well as of transport velocity. For trees, it has been shown that mild and short drought events can even increase the amount of assimilates allocated to roots (Hommel *et al.* 2016), while only more severe water restriction causes a reduction in the amount and the velocity of transport (Ruehr *et al.* 2009). Since our labeling was applied only shortly after the first signs of stomatal response and a decrease in photosynthesis (Figure 5-3), we might postulate that a first reaction of both species is a switch in carbon allocation priorities. Stems received less new assimilates and were thus not able to produce storage reserves (*P. australis*) or even had to almost completely consume the starch stored (*T. latifolia*) (Figure 5-4, Figure 5-5), while roots were prioritized with new assimilates, suggesting their use for osmoregulation.

5.5.2. Reduced carbon transfer from roots to microorganisms was likely due to reduction of microbial activity via direct drought effects

The ^{13}C excess values were lower in PLFAs from the drought treatment, which reveals either a reduced availability of plant-derived carbon for sediment microorganisms and/or a decreased activity of the microbial communities that depend on the rhizodeposited carbon. However, even though constricted, the carbon continuum between plants and the sediment remained in principle intact, as labeled carbon still reached the microbial community. It is, however, notable that reduced label incorporation into the PLFAs under drought was accompanied by higher $\delta^{13}C$ in the fast-turnover water-soluble organic matter fraction in the roots of both species. Therefore, on the one hand, the high priority for osmotic regulation might have prevented the carbon exudation by the roots under drought. On the other hand, it is possible that the direct impact of drought stress on the microorganisms reduced their metabolic activity and growth, causing reduced incorporation of labeled carbon into PLFAs. In this study, we found that not only less ^{13}C was incorporated into the PLFAs, but also a delay occurred, thus, supporting the idea that only a subset of the community was

active. It is also possible that sediment microorganisms were restricted under drought to particular water-containing microhabitats, as suggested by Schimel *et al.* (2007) and Moyano *et al.* (2013). Fuchslueger *et al.* (2014) assumed that bacteria in these disconnected microsites might be separated from root exudates. However, in contrast to these authors, we also observed a strong reduction of ^{13}C incorporation into the fungal biomarker c18:2ω6. Fungi are assumed to cope relatively well with drought stress, a behavior often attributed to their ability to better spatially explore the soil or sediment for water and nutrients (Frey *et al.* 2008; Riah-Anglet *et al.* 2015). We can, however, assume that in the sediments we used in our experiments, which originated from (under the current climate conditions) permanent water bodies, the fungal community was not adapted to strong desiccation. Only one PLFA (i16:0) displayed higher excess values in the drought compared to the control treatment. This PLFA is a marker for gram-positive bacteria, which have a thick, interlinked peptidoglycan cell wall and are therefore considered to have a stronger tolerance to desiccation (Schimel *et al.* 2007; Lennon *et al.* 2012; Fuchslueger *et al.* 2014). Nevertheless, we can confirm hypothesis (2), since, for the great majority of PLFA biomarkers, sediment desiccation caused a decrease in ^{13}C incorporation. Also, even though our experiment did not include a test of direct effects of drought, there were indications that the reduction of microbial activity was due to direct drought effects rather than a decrease in root exudation or exudate availability.

Plants and microorganisms are generally tightly linked and depend on each other, as plants affect microorganisms through carbon supply (Walker *et al.* 2003) and microorganisms, in turn, alter nutrient availability and cycling (Meier *et al.* 2015). While the plant internal carbon transport was adjusted, most likely to maintain osmoregulation in the roots of the two macrophytes examined, the plant-microorganism carbon continuum was clearly disrupted (but not totally blocked) by drought, most likely due to desiccation induced impairment of the microbial activity. Further studies are needed to assess the impact of such changes in the carbon continuum on plant and microbial community functioning and on the carbon and nutrient balance in sediments of small aquatic ecosystems and littoral zones. Due to their importance for the global carbon balance (Cole *et al.* 2007; Tranvik *et al.* 2009; Aufdenkampe *et al.* 2011), a deeper understanding of the functioning of inland freshwater ecosystems in a changing climate is necessary.

5.6. Acknowledgments

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6. Discussion

6.1. Overview

How ecosystems will change due to extreme climate events depends on the resistance and recovery mechanisms – or the disturbance-induced mechanisms and processes – of the single components of the ecosystem, but also on the strength of their interactions (Frank *et al.* 2015). Thus, estimating the strength of plant-microbe interactions is important to evaluate the ecosystem resistance to future climate change and increased drought occurrence. However, assessing changes in the coupling between microbial communities and plants under stress is difficult and response mechanisms are poorly understood, partly due to the need of interdisciplinary and labor-intensive approaches that combine plant and soil microbial expertise. Therefore, current research rather focusses on either plants or microbes instead of looking at interaction patterns under stress (Hueso *et al.* 2012; O'Brien *et al.* 2014; Butterfield *et al.* 2016; Hommel *et al.* 2016; Weise *et al.* 2016).

In the first experiment I focused on heat and drought effects on the understory plant and microbial communities of a beech forest (Figure 6-1a, b), because European beech is a dominant tree species in Central Europe but is known to be susceptible to drought (Geßler *et al.* 2007; Zang *et al.* 2014; Bolte *et al.* 2016). Extreme droughts can disturb the structure, composition, and functioning of forest ecosystems and the carbon cycling (Frank *et al.* 2015) which can lead to reduced vegetation productivity and depleted carbon stocks of plants (Reichstein *et al.* 2013; Frank *et al.* 2015; Anderegg *et al.* 2016). However, in my experiment, even under extreme heat and drought, the within plant transport of carbon was not completely disrupted and carbon sinks belowground were still active. Overall, I could show that, even though the implied stress could be considered as extreme (Smith 2011), the link between plants and microbes was not completely severed. Instead, I found strong alterations in the linkage of above- and belowground processes in the different treatments, with heat-drought having the strongest and heat the least negative effects. In summary, the carbon transfer of the examined beech forest understory ecosystem was severely affected under drought but the carbon continuum remained intact. Also, the stressed soil microbial community rather responded to changes in the linkage between above- and belowground carbon allocation and, in addition, showed a relatively high resistance under heat (H) and drought (D). Only the heat-drought (HD) treatment was severe enough to induce a strong reaction of the soil microorganisms by pushing the community to a limit where it responded with a community shift as well as a decrease in activity, diversity, and abundance.

In the second experiment I focused on drought effects on two emergent aquatic macrophytes, *Phragmites australis* and *Typha latifolia* (Figure 6-1c, d), that were grown on kettle hole sediment, in order to understand the effects of drought on the labile balance of aquatic ecosystems in terms of above- and belowground carbon coupling. Research on drought effects on the plant-sediment carbon transfer in aquatic ecosystems is scarce and only a few studies have covered emergent aquatic macrophytes (Li *et al.* 2004; Pagter *et al.* 2005), despite their relevance for aquatic-terrestrial ecosystem functioning (Downing *et al.* 2006; Werner *et al.* 2013). While well adapted to flooding, aquatic macrophytes are often assumed to be susceptible to drought (Li *et al.* 2004), where they have to cope with reduced carbon assimilation and water availability, which ultimately leads to carbon starvation and hydraulic failure (McDowell *et al.* 2011; Mitchell *et al.* 2013). With plants severed through stress, the plant-soil microorganism continuum, from which important ecosystem functions result, can be disrupted (Bardgett *et al.* 2005; Evans & Wallenstein 2014). This is especially relevant for small freshwater ecosystems like ponds and kettle holes (small, shallow standing freshwater systems), where emergent macrophytes play a central role in the system's carbon balance and where prolonged dry periods can lead to shifts from permanent to temporary water bodies (Werner *et al.* 2013; Reverey *et al.* 2016). Overall, I could show that the two examined aquatic macrophytes coped relatively well with the extreme drought stress. Also, drought induced a switch in plant carbon allocation priorities and, even though carbon cycling was strongly affected, the link between plants and microbes was still intact which is comparable to the analyzed understory system.

I did not include a heat treatment for the kettle hole experiment but instead investigated two different plant species under drought. In the beech forest monolith experiment, I conducted a 16S rRNA analysis but no NSC measurements, and vice versa in the kettle hole experiment.

Figure 6-1: Pictures of a) a Control monolith, b) a Heat-drought monolith, c) *P. australis*, d) *T. latifolia*

6.2. Heat and/or drought effects on the link between beech forest understory and soil microbial communities

6.2.1. Heat and/or drought effects on a beech forest understory

By analyzing changes in the leave and stem ^{13}C concentration before and after labeling, it became evident that, despite the strong stress, plants were assimilating carbon in all treatments (Figure 4-2a). However, the amount of recently assimilated carbon varied, with control > heat > drought 1 day after labeling (2 weeks of drought). In conclusion, a soil moisture deficit rather than temperature affected the carbon continuum of the investigated beech forest understory. The stronger effect of soil moisture compared to temperature has been shown in several studies, however, the severity of drought stress is strongly enhanced under increased temperatures due to a positive feedback loop where reduced cloudiness under drought leads to increased transpiration and thus a faster soil drying (Reichstein *et al.* 2013; Teskey *et al.* 2014; Ruehr *et al.* 2015; De Boeck *et al.* 2016).

Even though the plants were assimilating carbon and the increase in ^{13}C was significant in all treatments one day after labeling when compared to natural abundance, the allocation of assimilated carbon to the roots was severely inhibited in the heat and/or drought treatments (Figure 4-2b). Only in the control the increase in ^{13}C in the roots after labeling was significantly higher when compared to natural abundance. Nevertheless, even though not significant, an increase of root ^{13}C in the heat and/or drought treatments was detected, which indicates a carbon transfer despite the plants being severely stressed and it can thus be concluded that the connection to the belowground soil communities through carbon allocation was weakened but maintained.

6.2.2. Heat and/or drought effects on the soil microbial community of a beech forest understory

Through the analysis of the ^{13}C incorporation into PLFAs, it is possible to investigate those microbes that are tightly linked to the plants because an increase of ^{13}C in the PLFAs indicates that the microbes are feeding on carbon that was released into the soil from the plant roots via rhizodeposition. Furthermore, it is possible to investigate the active microbial community because only active microbes will consume labeled carbon and integrate it into their PLFAs. Thus, ^{13}C analyses in PLFAs can give insights on the active, rhizo-dependent soil microbial community.

By using this approach it could be demonstrated that the microbes closely related to plants were able to consume carbon even under strong environmental stress and that the heat-drought (HD) treatment led to the strongest decrease of ^{13}C in PLFAs, thus applying the strongest pressure on the active, rhizo-dependent microbial community where plants were also most severely affected (Figure 4-3).

The analysis of 16S rRNA offers the opportunity to detect changes in community structure, phylotype abundance, diversity, and species richness of the whole active bacterial community under environmental stress. When looking at shifts in the community structure (Figure 4-4), the bacterial community adapts to stress relatively fast. This is indicated by the strong initial community structure shift between DD 0 and DD 14 for D and HD, followed by a similar community structure at DD 28. Thus, it appears that the community reached a stable state (Scheffer & Carpenter 2003; Shade *et al.* 2012) after 2 weeks of drought in which it remained until the end of the experiment.

When looking at bacterial phylotype abundance, there was no shift in the H treatment (Figure 4-5), strengthening the proposed strong resistance theory of microbes under heat (Castro *et al.* 2010; Schindlbacher *et al.* 2011). Under drought, only *Planctomycetes* were negatively affected and decreased in abundance while other phyla did not change or even increased their abundance, supporting the conclusion of a dry optimum of many phyla as suggested by Lennon *et al.* (2012). Thus, only the HD treatment pushed the bacterial community as far as to a strong decrease in abundance for many phyla (Figure 4-5). Interestingly, shifts in the abundance of bacteria were phylogenetically highly conserved. For example, *Planctomycetes* generally reacted to the D and HD treatment with a decrease in abundance, *Actino*- and *Acidobacteria* only decreased under heat drought, and *Proteobacteria* even increased their abundance under those treatments. Thus, the moisture niche of soil microbes seems to be highly conserved because contrasting ecological strategies (e.g., dry-adapted generalists vs. wet-adapted specialists) can have a phylogenetic signal at a coarse taxonomic level (Lennon *et al.* 2012; Barnard *et al.* 2013).

6.2.3. Heat and/or drought effects on the link between beech forest understory and soil microbial communities

Combining PLFA and 16S rRNA results it can be assumed that under the heat and/or drought treatments the microbial community of a beech forest understory shifted to a more plant-independent community because the stress rather affected rhizo-dependent microbes. For H and D on the one hand, the PLFA results indicate that there is a reduced activity in the rhizo-dependent microbes, while there is no significant change in the diversity and species richness of the whole bacterial community. And for HD on the other hand, where plants were most severely affected, a decrease in the bacterial diversity and abundance – and the lowest level of label in the PLFAs – was observed. Both findings indicate that microbes rather respond to the indirect plant-mediated effects of drought stress, e.g., through a reduced carbon transport to roots and reduced rhizodeposition. Thus, while many studies focus on direct drought effects on microbes (Berard *et al.* 2011; Manzoni *et al.* 2012; Hueso *et al.* 2012) the indirect effects mediated through plants might actually be more important in terms of microbial community composition and function (Classen *et al.* 2015).

6.3. Drought effects on the link between emergent aquatic macrophytes and kettle hole sediment microbial communities

6.3.1. Drought effects on emergent aquatic macrophytes

Even though drought altered the rates of photosynthesis (A) and stomatal conductance (g_s) of the two tested aquatic macrophytes, they displayed a high tolerance with stable rates for both parameters until around 10 days of ongoing strong drought when the rates started to decreased (Figure 5-3).

Non-structural carbon (NSC) concentration variations through drought stress led to different responses in the two plant species. *P. australis* leaves were relatively unaffected in terms of total NSC concentrations and there was an increase of NSCs in both control and drought over time in the stems (Figure 5-4). In *T. latifolia*, leaves and stems acted similar and displayed a decrease in NSC contents in response to drought (Figure 5-5). A reduced assimilate export from and increased sugar accumulation in leaves have been linked to the adjustment of the osmotic potential, leading to changes in the NSC concentration in plants (Sánchez *et al*., 1998; Pagter *et al*., 2005; Peuke *et al*., 2006). NSCs are needed for regulating hydraulic functioning and osmotic potential. This positive relationship between NSCs and drought tolerance was, for example, found in tropical tree seedlings (O'Brien *et al*., 2014). However, with no increase in leaf sugars over time, it can be assumed that osmoprotection in the leaves did not occur for these two wetland species under the imposed drought. Still, including the ^{13}C label results, it was shown that stem tissues received less new assimilates, while roots were prioritized with new assimilates (Figure 5-7, Figure 5-8), suggesting their use for osmoprotection. Therefore, the tested emergent aquatic macrophytes seem to either prioritize root survival over leaves under drought stress or the initial osmotic stress was more pronounced in the roots. The first assumption fits the general survival strategy of the two species as they both form extensive belowground rhizomes that allow regeneration of the shoots (Brix *et al.* 2001; Hartzendorf & Rolletschek 2001; Kercher & Zedler 2004; Engloner 2009).

It seems that the drought stress did not affect carbon uptake of the leaves until after approx. 2 weeks of drought because there were no significant differences in the ^{13}C incorporation between control and drought in both species one day after labeling (Figure 5-6). This could be due to the fact that the drought stress was not affecting the plants strong enough at the time of labeling. However, as the drought progressed, photosynthesis, as well as stomatal conductance started to decline indicating a stress response to the reduced water availability. Thus, both wetland species were able to cope with a longer duration of drought (at least 2 weeks) before they showed signs of stress. Nevertheless, the amount of recently assimilated carbon that was transported from leaves to other plant compartments was altered under drought conditions in *P. australis* as well as in *T. latifolia* (Figure 5-7, Figure 5-8).

In summary, the analyzed emergent aquatic macrophytes were relatively resistant to drought. This could be an adaptation of the plants to kettle hole ecosystems and to the situation close to freshwater shorelines in general. These systems easily dry out when water levels lower, which could imply that wetland species that grow at terrestrial-aquatic interfaces have a higher drought tolerance than previously thought (Kercher & Zedler 2004; Li *et al.* 2004).

6.3.2. Drought effects on the link between two emergent aquatic macrophytes and kettle hole sediment microbial communities

Even under strong drought stress, the recently assimilated carbon that was transported to the sediment via root exudation reached microbial PLFAs. This was evidenced by the increase of ^{13}C in the PLFAs after labeling. However, the linkage between above- and belowground communities was weakened – indicated by lower ^{13}C values in the drought stressed microbial PLFAs – maybe due to a reduced availability of plant-derived carbon for sediment microorganisms and/or a decreased activity of the microbial communities that depend on the rhizodeposited carbon.

Even though there was an accumulation of ^{13}C-labeled sugars in the roots, that was only slightly lower in the drought treatment compared to the control, there was a delay of ^{13}C incorporation into the PLFAs of sediment microorganisms under drought – indicated by the ^{13}C peak in the PLFAs that occurred later in the drought treatments when compared to the control – which points to a reduced microbial activity.

Thus, it can be assumed that a reduction of microbial activity via direct drought effects rather than a reduced plant root exudation led to reduced ^{13}C incorporation into the PLFAs. Direct drought effects e.g., through desiccation of the sediment and radiation, can lead to impaired physiological properties of microbial membranes, proteins, and nucleic acids. Also, microbes prevent dehydration by intracellular lowering of the water potential through osmolyte accumulation, but this process reduces microbial growth and functioning due to a high energy demand (Schimel *et al.* 2007; Reverey *et al.* 2016).

6.4. Ecosystem reactions under drought stress

Forests are important terrestrial carbon sinks because they store around 45% of the carbon found in terrestrial ecosystems (Anderegg *et al.* 2012; Reichstein *et al.* 2013). However, when they are exposed to extreme climate events like droughts, they alter their use and allocation of nutrients and carbon. Reduction in the biomass carbon sink and widespread increases in tree mortality may dominantly be caused by water stress through droughts which alter the balance between plant photosynthesis and respiration (Peng *et al.* 2011; Anderegg *et al.* 2012, 2016; Ma *et al.* 2012). This leads to reduced carbon uptake and accumulation rates and thus higher CO_2 concentrations in the atmosphere. Thus, droughts can reduce the net primary production (NPP), net carbon exchange, gross primary productivity, and the carbon sequestration of forests. Estimates of long-term trends of the NPP show a decline in recent years and under the future global climate, we can expect a further NPP reduction (Schlesinger *et al.* 2016). Severe droughts can even turn a forest into a carbon source (Ciais *et al.* 2005; Ma *et al.* 2012; Schlesinger *et al.* 2016).

Next to forests, small water bodies like kettle holes may seem to play an insignificant role in terms of carbon cycling. However, due to their large surface area – which was greatly underestimated in the past – and high carbon processing activity, small water bodies may actually be among the most important ecosystems for organic carbon sequestration (Downing 2010; Reverey *et al.* 2016). Since small water bodies tend to be more heterotrophic with higher surface CO_2, but lower O_2 concentrations, their greenhouse gas (GHG) emissions and carbon sequestration rates are higher when compared to larger water bodies. As a result, it can be assumed that small water bodies constitute at least a third of the processing by aquatic ecosystems worldwide and their carbon sequestration rates may thus be equal or greater than any other ecosystem in the world including forests, grasslands, and oceans (Downing 2010). With the projected increase in droughts in the future, small water bodies will be subject to increasing water level fluctuations and longer and more frequent periods of falling dry. Under drought, water levels decrease through increased evapotranspiration and aerobic conditions are more frequent, which affects carbon turnover, GHG releases, and sediment microbial community composition and activity – for example through shifts from anaerobic to aerobic microbes or increases in CO_2 emissions under sediment desiccation through higher diffusion rates in the air when compared to water (Reverey *et al.* 2016; Weise *et al.* 2016).

When comparing the two investigated systems in terms of drought resistance (Table 6-1), it is notable that they both exhibited a relatively strong carbon linkage between above- and belowground communities and even under extreme environmental stress the carbon continuum remained intact in both systems even though weakened. Also, the aquatic aboveground communities seemed to cope better with drought, when compared to the forest understory plants, when looking at the ^{13}C

allocation between different plant compartments. Interestingly, the opposite was the case in terms of the belowground microbial communities. While soil microbes displayed a strong resistance to environmental stress and were rather indirectly influenced by the stress through the plants, sediment microbes appeared to be rather sensitive to drought stress and apparently suffered directly from it. However, a direct comparison of the drought effects in the two investigated systems is difficult because the drought conditions varied. Due to the numerous variables that have to be considered when looking at drought effects on ecosystems – e.g., the severity and duration of the drought, biome type, morphology, phenology, sensitivity, and physiology of the plants, prevailing climatic conditions, and species composition – drought impacts are hard to evaluate (Gazol *et al.* 2016; Schlesinger *et al.* 2016). Thus, since ecosystems react nonlinearly, a comparison of effects of climate extremes on the carbon cycle is difficult (Frank *et al.* 2015).

Whether drought influences microbes directly (e.g., through osmotic stress) and/or indirectly (e.g., through altered carbon supply from plants) is still uncertain but it can be assumed that indirect effects play a greater role than previously assumed (Classen *et al.* 2015). If indirect effects are the cause for stressed microbes, then there should be a correlation between microbial community shifts and the physiological reactions of the aboveground vegetation. Since the greatest changes of the microbial community, as well as the least integration of ^{13}C into PLFAs, appeared in the HD treatment of the beech forest understory system, where plants were most severely affected, it could be inferred that microbes rather suffered from indirect plant related stress than from direct drought stress. However, when looking at how a changing microbial community alters its functional rates, for example carbon uptake, functional redundancy hinders concrete conclusions. In a community with a high functional redundancy, where functions are carried out by many taxa, community shifts will not ultimately lead to changes in functional rates, whereas a low redundancy increases the sensitivity of the performed function and thus closely follows microbial abundance changes (Shade *et al.* 2012). In the aquatic kettle hole sediment system, the high priority for osmotic regulation of plants might have prevented the carbon exudation from roots under drought, leading to a reduced availability of plant-derived carbon for sediment microorganisms. On the other hand, and more likely, it is possible that the direct impact of drought stress on the microorganisms reduced their metabolic activity and growth, causing reduced incorporation of labeled carbon. Shifts in the microbial community composition can lead to alterations in the ecosystem functioning, especially when organisms that regulate specific processes like decomposition of SOM, (de)nitrification, or methanogenesis are affected. However, processes that are carried out by numerous microbes will rather be affected by direct impacts like soil moisture than by microbial community composition (Classen *et al.* 2015).

Table 6-1: Comparison of drought effects on the two analyzed ecosystems.

	Beech forest understory	Aquatic kettle holes
Plant C allocation	Reduced transfer to roots	Reduced transfer to stems, increased in roots
Drought effects on microbes	Rather indirect through plants	Rather direct through drought
PLFAs	Carbon linkage not completely disrupted → ^{13}C reached microbial PLFAs	Carbon linkage not completely disrupted → ^{13}C reached microbial PLFAs
PLFAs	Reduced ^{13}C incorporation into PLFAs under stress	Reduced ^{13}C incorporation into PLFAs under stress
Microbes	Relatively resistant	Relatively sensitive

6.4.1. How will (beech) forests react under future climate change?

When looking at forest ecosystems under drought, Reichstein *et al.* (2013) named some specific impacts which include the effect of water availability on plant physiology, phenology, and carbon allocation patterns, as well as shifts in the vegetation composition with impacts being large and delayed due to the longevity of trees. Climate change, on the one hand, leads to an increase in temperatures and a decrease in precipitation which has a long-lasting effect on forests and e.g., affects carbon relations and tree species distribution (Granier *et al.* 2007; Hanewinkel *et al.* 2012). On the other hand, extreme climate events like strong droughts are increasing which leads to severed plant-soil carbon relations and potential long-term effects like forest die-offs (Mcdowell *et al.* 2008; Allen *et al.* 2010). Extreme droughts are key drivers of vegetation change by negatively affecting tree physiological responses and accelerating biotic attacks, which can lead to tree mortality (McDowell *et al.* 2011; Allen *et al.* 2015; Anderegg *et al.* 2016). The consequences of a disrupted forest ecosystem for biodiversity, ecosystem structure, and function, as well as ecosystem services are huge. Forests accumulate huge amounts of anthropogenic CO_2 and are a major terrestrial carbon sink that stores around 45% of the terrestrial carbon, thus mitigating the atmospheric CO_2 increase (Friedlingstein *et al.* 2001; Ciais *et al.* 2005; Bonan 2008; Reichstein *et al.* 2013).

Given the results of this study, it can be expected that the understory of the investigated beech forest – that also comprises the cradle of the future forest, i.e. the tree natural regeneration – will cope with extreme droughts through the strong linkages of above- and belowground communities and shifts in the community structure to more adapted species. Thus, I demonstrated that a strong drought event could not completely disrupt the carbon continuum of a beech forest understory pointing to a relatively high tolerance in the short-term. However, the whole beech forest understory was investigated and long-term effects were not determined. Thus, even though the understory and soil communities showed a relatively high tolerance towards heat and drought, beech trees, in general, are susceptible to drought (Geßler *et al.* 2007; Bolte *et al.* 2016), and how changes in understory and soil communities in addition to drought stress will affect them is unclear.

Beech trees are susceptible to drought because of their conservative shade-tolerant growth strategy and may suffer from drought-induced xylem embolism and reduced nutrient uptake and growth (Geßler *et al.* 2007; Robson *et al.* 2009). Beech forests as temperate deciduous forests common in Central Europe are rarely exposed to extreme droughts and may thus lose larger fractions of their range at the cost of more drought-adapted species like oaks or coniferous stands with pine (Geßler *et al.* 2007; Granier *et al.* 2007; Hanewinkel *et al.* 2012). For example, beech trees in Europe are predicted to change their range from today's ranges in western Europe (France, Netherlands, Germany) and the lower elevations in central and eastern Europe more to central, northern and northeastern Europe (Hanewinkel *et al.* 2012). However, it is suggested that mixed stands, for instance with oak, might improve the drought resistance of beech trees (Leuschner *et al.* 2001; Metz *et al.* 2016).

Also, because beech trees have a shade-tolerant growth strategy the seedling response in the forest understory is an important factor of beech survival under future climate change. Increasing summer droughts have the potential to reduce the growth and competitive ability of beech seedlings – due to the stress of simultaneously coping with water limitations and tolerating shade – which can impact the natural regeneration of beech forests (Geßler *et al.* 2007; Robson *et al.* 2009; Bolte *et al.* 2016). Thus, the response of the understory vegetation plays an important role in terms of (beech) forest resistance and regenerative abilities and therefore, further research on long-term effects of extreme drought events on the understory and microbial communities is necessary.

6.4.2. How will small aquatic systems react under future climate change?

Small scale aquatic ecosystems play a more significant role than previously thought when looking at carbon rates and processes and their importance for the global carbon cycle under future climate scenarios should not be underestimated (Downing *et al.* 2006). With impending climate change, kettle holes will be pressured by stronger water level fluctuations in their littoral zones, to the point of desiccation of the kettle hole (sediment) over longer time periods with shifts from permanent to temporary water bodies (Reverey *et al.* 2016; Weise *et al.* 2016). Given the results of this study it can be assumed that typical kettle hole plants like *P. australis* and *T. latifolia* cope with the drought stress through carbon allocation to the roots for osmoprotection and that they can withstand even strong desiccation of the sediment in the short-term, while belowground microbial communities will reduce their activity but keep the linkage to aboveground plant communities intact. With the present study I could not detect shifts in the bacterial sediment community but Weise *et al.* (2016) conducted a 16S rRNA study on kettle hole bacterial communities under drought and found that "only the most extreme hydrological changes induced a significant shift in the active and total bacterial communities" supporting my findings that even aquatic ecosystems are in general relatively drought tolerant.

When looking at the two tested emergent aquatic macrophytes it becomes evident that both species are generalists in terms of hydrologic conditions. Common reed (*P. australis*) is a clonal, perennial species (Brix *et al.* 2001; Pagter *et al.* 2005; Engloner 2009) that is well adapted to wet environments due to a high air space amount and to different convection methods that improve the oxygen availability of the rhizome and the efflux of oxygen from roots to the rhizosphere (Engloner 2009). However, reed has also adapted to dry periods with its high intrinsic water use efficiency and the ability to photosynthesize under drought (Pagter *et al.* 2005). Due to its worldwide distribution, reed dominated wetlands may have a considerable effect on climate change as they can function as a GHG sink over longer time scales (Brix *et al.* 2001; Engloner 2009). Cattail (*Typha latifolia*) also is an important species of freshwater wetlands worldwide (Li *et al.* 2004). It is an erect perennial semi-aquatic graminoid, is capable of spreading large distances via seed and rhizome fragments, and can form dense monotypic stands with few coexisting species. Being tall and productive, cattail can cope with a variety of hydrologic conditions and copes with drought via down-regulation of photochemistry and decreased photosynthesis, growth, and stomatal conductance (Li *et al.* 2004). In other words, it can outcompete habitat specialists due to a high stress tolerance and can, therefore, be considered as an invasive plant even if native under future climate scenarios (Kercher & Zedler 2004). Thus, both tested species could play a significant role under future climate change due to a relatively high drought tolerance and worldwide contribution. Thus, instead of being negatively affected by drought the two investigated species could actually profit by outcompeting and replacing other species that are more susceptible to drought.

6.5. Conclusion and outlook

The present study assessed plant-microbe interactions under heat and/or drought stress in two climate-threatened ecosystems, in order to understand what regulates the strength of plant-microbe linkages under different environmental stressors and in different ecosystems. In a nutshell, I could demonstrate that the investigated ecosystems, a beech forest understory and an aquatic kettle hole sediment system, have a relatively high tolerance against extreme events like drought, at least in the short-term, partly due to the strong plant-soil/sediment microorganism carbon continuum, which was weakened but not severed under the induced heat and/or drought stress. I conclude that ecosystems strongly depend on and try to maintain a functional plant-soil/sediment microorganism carbon continuum under drought, which might help to withstand the increase in extreme drought events under future climate change.

I infer that the response of the understory vegetation plays an important role in terms of (beech) forest resistance and regenerative abilities and therefore, further research on long-term effects of extreme drought events on the understory and microbial communities and the subsequent effects on the distribution of forests is necessary. Also, I propose that both tested wetland species – instead of being negatively affected by drought – could actually profit by outcompeting and replacing other species due to a relatively high drought tolerance and worldwide contribution. Therefore, further research on the drought stress responses and mechanisms of kettle hole ecosystems is necessary to evaluate whether a biodiversity loss in favor of drought-tolerant aquatic species occurs under future climate change and how this affects the systems' carbon dynamics.

In general, further research on the recovery of the tight link and community structures in different ecosystems after extreme climate events is required to evaluate the damage that they induce in a wider timeframe in terms of, amongst others, biodiversity loss, species distribution, and carbon cycling. As ecosystems are highly complex and respond to stress in numerous ways, several stable states may exist, in which a community can reside. Under stress, a community can (a) resist without great changes, (b) change but go back to the original state (resilience) or (c) shift to a new stable state and gain a different equilibrium from the previous state which might be more adapted to the same stress in the future (Shade *et al.* 2012). My experimental design excluded the possibility to test whether the analyzed microbial community will return to its original community structure and activity (resilience) or if there is a permanent shift to an alternative stable state. However, a rewetting study after an extreme drought experiment could give insights on the recovery of the system and consequences for the carbon cycle.

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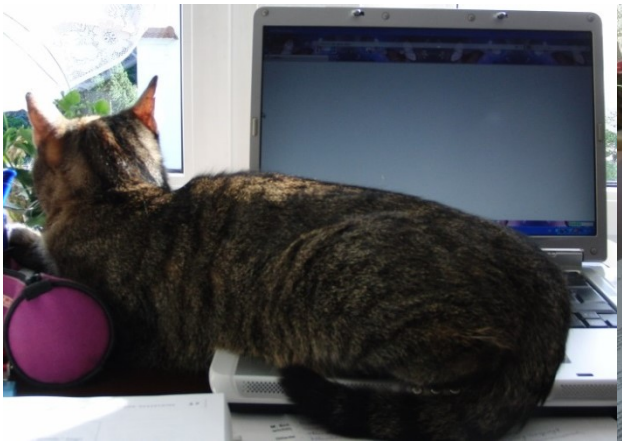

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Selbstständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit selbstständig und ausschließlich unter Verwendung der angegebenen Quellen sowie ohne unzulässige Hilfe Dritter angefertigt habe. Diese Doktorarbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form oder auszugsweise einer Prüfungsbehörde vorgelegt.

Berlin, den 15.03.2017

Isabell von Rein

Appendix

Table S 1: Information on plant species found on the monoliths. Monoliths are separated into treatments (C, D, H and HD) with n = 5. Values represent the number of monoliths where the plant species was present.

Plant Species	Control	Drought	Heat	Heat-Drought	Total
Acer pseudoplatanus	3	2	-	1	5
Fraxinus excelsior	3	5	3	4	15
Oxalis acetosella	3	3	3	5	14
Galium odoratum	5	5	5	2	17
Melica uniflora	2	4	1	3	10
Fagus sylvatica	2	2	2	4	10
Lamiastrum galeobdolon	4	4	2	4	14
Carpinus betulus	1	-	1	-	2
Acer platanoides	-	-	1	1	2
Impatiens parviflora	-	1	-	-	1
Hordelymus europaeus	3	1	-	1	5
Carex sylvatica	-	2	-	1	3
Stellaria holostea	2	1	2	-	5
Mercurialis perennis	2	-	-	1	3
Athyrium filix-femina	-	1	2	-	3
Geranium robertianum	1	-	1	1	3
Milium effusum	-	2	1	-	3
Dentaria bulbifera	-	-	1	1	2
Urtica dioica	-	-	1	-	1
Allium ursinum	1	-	-	-	1

Table S 2: Phopholipid fatty acids (PLFAs) that were used as markers for certain groups of microorganisms in the experiment.

PLFA	Marker for
c14:0	general bacteria
c15:0	general bacteria
c16:0	general bacteria
c16:1ω9c/7c	general bacteria
c17:0	general bacteria
c18:0	general bacteria
c18:1ω9t/7c	general bacteria
i16:0	gram+
i17:0	gram+
a17:0	gram+
a15:0	heterotroph
i15:0	heterotroph
10Me17:0	actinomycetes (gram+)
10Me18:0	actinomycetes (gram+)
c16:1ω5c	arbuscular mycorrhizal fungi
c18:1ω9c	fungi

Table S 3: pH and O_2 (mg l^{-1}) from the tub water of the controls and pH from the soil of the drought treatments dissolved in distilled water. Values are means (n = 6) ± SE.

Days after D	-5	0	6	10	14	23	24
pH							
PC	8.6±0.1	8.4±0.1	8.6±0.1	8.6±0.1	8.5±0.1	8.7±0	8.7±0
PD	8.5±0.1	8.2±0.2	7.8±0	7.9±0	7.8±0	7.1±0.1	-
TC	8.7±0	8.2±0.1	8.6±0.1	8.6±0.1	8.5±0.1	8.6±0	8.7±0
TD	8.7±0.1	8.2±0.1	7.6±0.1	7.6±0.1	7.5±0	6.9±0.1	-
O_2							
PC	7.1±0.2	6.2±0.2	7.1±0.2	6.9±0.7	8.2±0.2	8.1±0.2	7.6±0.2
TC	7.4±0.2	6.1±0.3	7.5±0.2	7.6±0.2	8.1±0.4	9.0±0.7	8.1±0.1

Table S 4: Phospholipid fatty acids (PLFAs) that were used as markers for certain groups of microorganisms in the experiment.

PLFA	Marker for
c16:0	general PLFA found in both bacteria and algae
c16:1ω9c/7c	general microbial biomarker
c18:0	general PLFA found in both bacteria and algae
c18:1ω9c	general PLFA found in both bacteria and algae
c18:1ω9t/7c	general bacteria
a15:0	heterotroph
i15:0	heterotroph
i16:0	gram+
Cy19:0	Gram-
10Me16:0	SRB and Actinobacteria
c18:2ω6	fungi

Figure S 1: Pictures of heat-pulse with drought (HD) monoliths

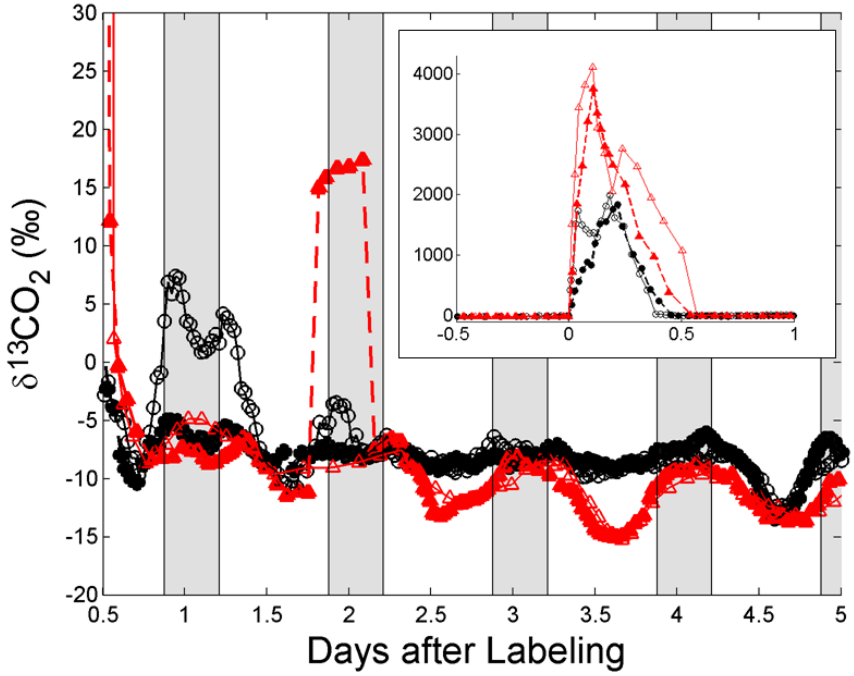

Figure S 2: $\delta^{13}CO_2$ values (‰) inside the chamber during labeling (inset graph) and gas within the soil for five days after labeling. C (open circle, solid line) and D (filled circle, dotted line) isotopic patterns are depicted by the black lines. H (open triangle, solid line) and HD (filled triangle, dotted line) are depicted by the red lines. Grey bars indicate evening hours. The inset graph is for displaying the $\delta^{13}CO_2$ patterns during labeling. The larger graph displays the treatments shortly after the label chamber was removed. During the labeling there was no separation made between soil and canopy air.

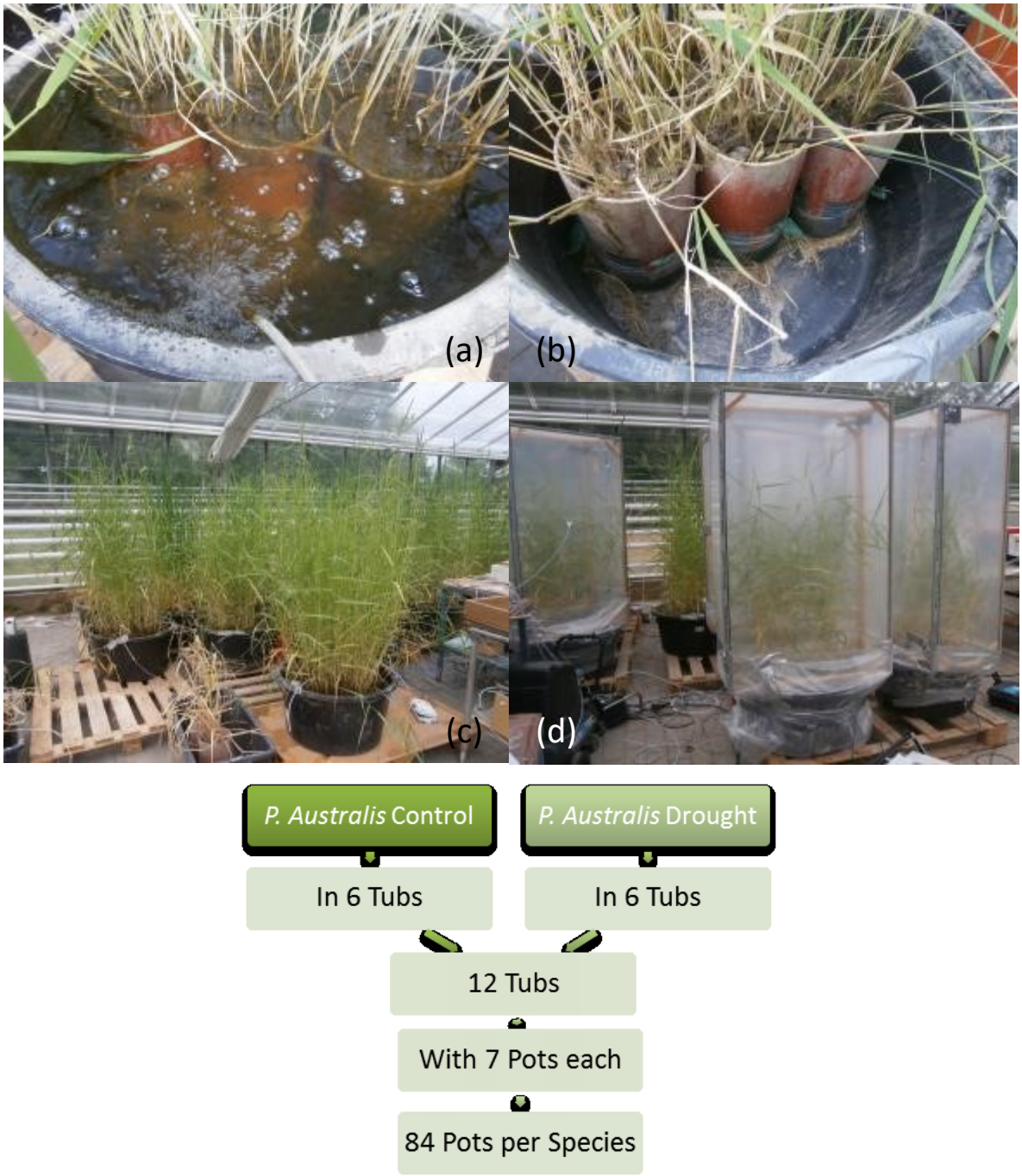

Figure S 3: Pictures of the macrophytes and scheme of the experimental design: close ups of (a) control and (b) drought tub, (c) Macrophytes (*P. australis* and *T. latifolia* (in the back)) at the beginning of the experiment, (d) *T. latifolia* in the gastight chambers during labeling, (e) shows the treatments and the number of pots for *P. australis* (the same design was applied to *T. latifolia*)

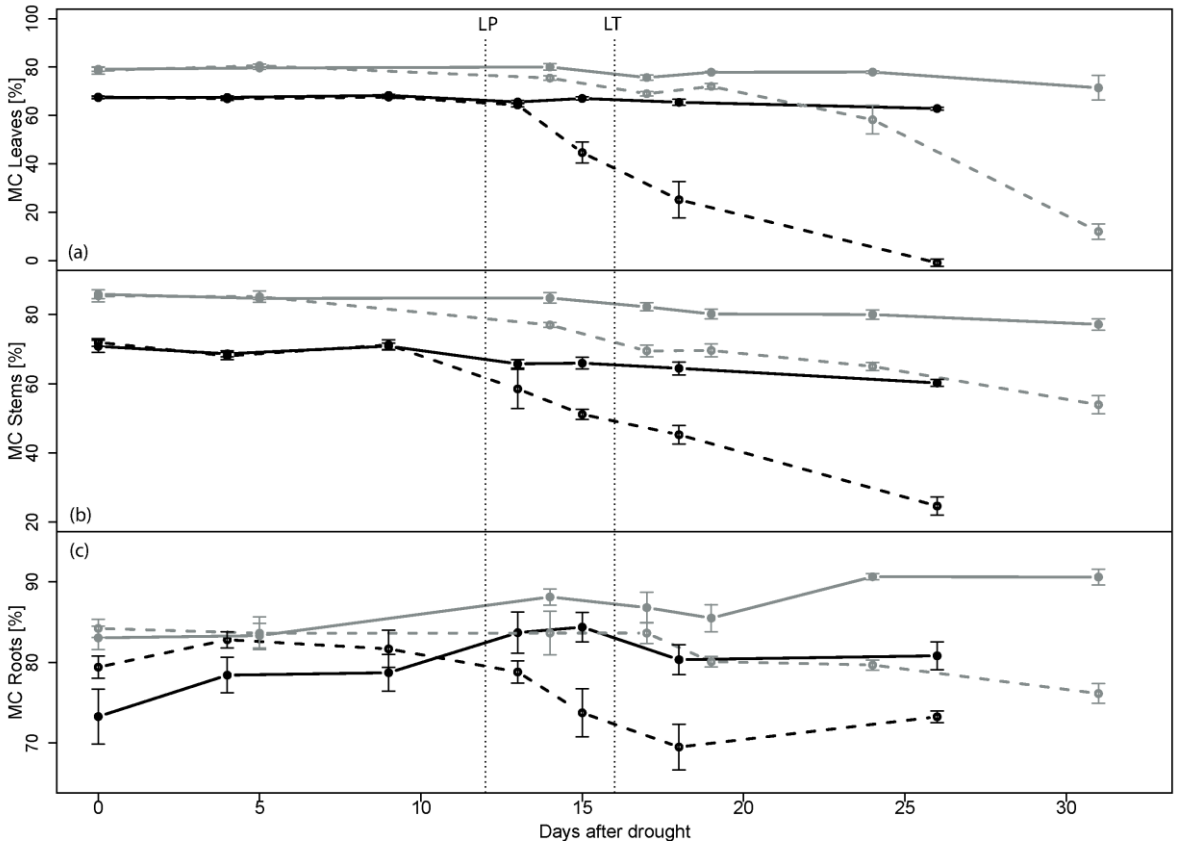

Figure S 4: Moisture Content (MC) [%] for leaves (a), stems (b) and roots (c) for *P. australis* (black) and *T. latifolia* (gray) for control (solid lines) and drought (dotted lines). Values are means (n = 6). Error bars indicate SE. LP and LT: time of ^{13}C pulse labeling for *P. australis* and *T. latifolia*, respectively.